

29 Aug 2019

Protocol Synopsis

Title	Effect of Dupilumab (anti-IL4R α) on the Host-Microbe Interface in Atopic Dermatitis
Short Title	Dupilumab Study
Clinical Phase	Post-Marketing Mechanistic Trial
Number of Sites	Up to 16 clinical sites in the United States
IND Sponsor/Number	Not Applicable
Study Objectives	<p>Primary Objective</p> <p>To assess the effect of dupilumab on <i>Staphylococcus aureus</i> (<i>S. aureus</i>) abundance on lesional skin as measured by microbial deoxyribonucleic acid (DNA)</p> <p>Secondary Objectives</p> <ol style="list-style-type: none"> 1. To assess the effect of dupilumab on <i>S. aureus</i> abundance on non-lesional skin as measured by microbial DNA 2. To assess the effect of dupilumab on epidermal barrier function in lesional and non-lesional skin 3. To assess the effect of dupilumab on disease severity <p>Exploratory Objectives</p> <ol style="list-style-type: none"> 1. To assess the effect of dupilumab on the microbiome and metagenome in lesional and non-lesional skin 2. To assess the effect of dupilumab on the skin transcriptome in lesional and non-lesional skin (non-URMC participants only) 3. To assess the effect of dupilumab on epidermal lipids in lesional and non-lesional skin 4. To assess the effect of dupilumab on the expression of <i>S. aureus</i> superantigens, toxins, lipase, and proteases on lesional and non-lesional skin 5. To assess the effect of dupilumab on non-lesional skin barrier structure and Langerhans cells (LC) by confocal imaging (URMC participants only) 6. To assess the effect of dupilumab on the function of the skin microbiome (i.e. ability of Coagulase-negative staphylococci isolates [CoNS] to kill <i>S. aureus</i>) in lesional and non-lesional skin

	<ol style="list-style-type: none"> To assess the effect of dupilumab on PBMC immunoprofiles. To assess the effect of dupilumab on serum biomarkers (e.g. Th2 biomarkers) To explore the association between genetic variation and (clinical and mechanistic) responsiveness to dupilumab To identify networks/pathways associated with responsiveness to dupilumab, quantified by disease severity, symptoms, and barrier function.
Study Design	A multi-center randomized double-blind, placebo-controlled (RDBPC) trial investigating the effect of 6 weeks of dupilumab treatment on quantitative and qualitative measures of cutaneous microbial community structure, skin barrier biology, and circulating T cell profiles, followed by a 10 week open-label extension.
Primary Endpoint(s)	<i>S. aureus</i> abundance as measured by microbial DNA (<i>femA</i> qPCR) on lesional skin at Day 28
Secondary Endpoints	<ol style="list-style-type: none"> <i>S. aureus</i> abundance as measured by microbial DNA (<i>femA</i> qPCR) on lesional skin at Days 0, 3, 7, 14, 21, 42, 77 and 112 <i>S. aureus</i> abundance as measured by microbial DNA (<i>femA</i> qPCR) on non-lesional skin at Days 0, 3, 7, 14, 21, 28, 42, 77 and 112 Basal (prior to tape stripping) transepidermal water loss (TEWL) of non-lesional and lesional skin at Days 0, 3, 7, 14, 21, 28, 42, 77 and 112 TEWL area under the curve of non-lesional skin at Days 0, 7, 14, 21, 28, 42, 77 and 112. TEWL will be assessed prior to tape stripping and repeated after 5, 10, and 15 tape strips. Change in TEWL per every 5 tape strips (i.e. slope) on non-lesional skin at Days 0, 7, 14, 21, 28, 42, 77 and 112 Eczema Area and Severity Index [EASI], Investigator Global Assessment [IGA], and SCORing Atopic Dermatitis [SCORAD] at Days 0, 3, 7, 14, 21, 28, 42, 77 and 112 Pruritus numerical rating scale [NRS] at Days 0, 3, 7, 14, 21, 28, 42, 77 and 112
Exploratory Endpoints	<ol style="list-style-type: none"> Composition of bacterial taxa in lesional and non-lesional skin at Days 0, 3, 7, 14, 21, 28, 42, 77 and 112 Abundance of bacterial taxa in lesional and non-lesional skin at Days 0, 3, 7, 14, 21, 28, 42, 77 and 112

	<ol style="list-style-type: none"> 3. Gene expression in the skin transcriptome in non-lesional skin at Days 0 and 7, and in lesional skin at Days 0, 7, and 21 4. Lipid profiles of non-lesional skin at Days 0, 7, 14, 21, 28, 42, 77 and 112 and of lesional skin at Days 0, 14, 28, and 112 5. Expression of <i>S. aureus</i> superantigens, toxins, lipase, and proteases on lesional and non-lesional skin at Days 0, 3, 7, 14, 21, 28, 42, 77 and 112 6. Confocal imaging of tight junctions (TJs) and relationship to LCs in the epidermis from non-lesional skin at Days 0, 7 and 21 7. Percent of coagulase-negative staphylococci [CoNS] isolates that kill <i>S. aureus</i> on lesional and non-lesional skin at Days 0, 3, 7, 14, 21, 28, 42, 77 and 112 8. PBMC immunoprofiling at Days 0, 14 and 28 9. Levels of serum biomarkers (e.g. Th2 biomarkers and anti-drug antibodies [ADA]) at Days 0, 7, 14, 21, 28, 42, 77 and 112 10. The presence of single nucleotide polymorphisms (SNPs)
Accrual Objective	This study will enroll approximately 99 adult participants, 18-75 years of age, with chronic moderate-to-severe atopic dermatitis (AD). A 2:1 allocation will be used to assign 66 participants to the dupilumab arm and 33 participants to the placebo arm.
Study Duration	This study will take approximately 20 months to complete; we anticipate that enrollment will take approximately 1 year.
Treatment Description	<p>RDBPC Portion</p> <p>Active: Dupilumab is a fully human monoclonal antibody (mAb) directed against the IL-4 receptor alpha subunit (IL-4Rα), which is a common subunit for the Type 1 and Type 2 receptors that mediate most of the biological functions of the prototypic Th2 cytokines, IL-4 and IL-13. Participants will receive a loading dose of two 300 mg subcutaneous injections on Day 0 followed by 300 mg subcutaneous injections every two weeks (Days 14 and 28). Injections can be given in the abdomen, thigh, or upper arm.</p> <p>Placebo: Placebo will contain the identical formulation as the dupilumab formulation without the active mAb and will be given by exactly the same route and schedule through Day 28.</p> <p>Open-label Extension Portion</p> <p>All participants will begin a 10 week open-label extension (OLE) on Day 42. Participants who were randomized to placebo initially will</p>

	<p>receive a 600 mg loading dose (two 300 mg injections) of dupilumab on Day 42. Those who were randomized initially to dupilumab will receive a 300 mg dupilumab injection plus a placebo injection on Day 42 to maintain the blind. Participants or their caretaker will self-administer 300 mg of dupilumab by subcutaneous injection every two weeks through Day 98. Injections will be given in the abdomen, thigh, or upper arm.</p>
Inclusion Criteria	<p>Individuals who meet all of the following criteria are eligible for enrollment as study participants:</p> <ol style="list-style-type: none"> 1. Must be able to understand and provide informed consent 2. Male or female, 18-75 years inclusive at the Screening Visit 3. Chronic AD, (according to the Atopic Dermatitis Research Network [ADRN] Standard Diagnostic Criteria), that has been present for at least 3 years before the Screening Visit for individuals aged 18-65 years old OR for at least 5 years before the Screening Visit for individuals aged 66-75 years old. Individuals aged 66-75 years of age must have documentation of a skin biopsy to rule out cutaneous T-cell lymphoma and other non-AD conditions present in the medical record. 4. EASI score ≥ 12 at the Screening Visit and ≥ 16 at the Treatment Initiation Visit 5. IGA score ≥ 3 (on the 0-4 IGA scale) at the Screening and Treatment Initiation Visits 6. $\geq 10\%$ body surface area of AD involvement at the Screening and Treatment Initiation Visits 7. Must have active lesions (minimum of 3 of at least $4 \times 4 \text{ cm}^2$ each on the upper or lower extremities or trunk, excluding the palms of the hands, soles of the feet, buttock, navel, axilla, suprapubic area, and neck) at the Screening and Treatment Initiation Visits 8. Documented recent history (within 6 months before the Screening Visit) of inadequate response to outpatient treatment with topical corticosteroids of medium to high potency (\pm topical calcineurin inhibitors as appropriate), or for whom topical treatments are otherwise inadvisable (e.g., because of important side effects or safety risks). Acceptable documentation includes contemporaneous chart notes that record prescription of topical corticosteroids and/or topical calcineurin inhibitors, investigator documentation based on communication with patient's treating physician, or medical history provided by the patient.

	<ol style="list-style-type: none"> 9. Must agree to apply a stable dose of a topical emollient (moisturizer) at least twice daily for at least 7 days before the Treatment Initiation Visit, and must confirm application at the Treatment Initiation Visit 10. Individuals with asthma must adhere to asthma controller medication(s) for the duration of the study including the open-label and follow-up portions of the study 11. Females of childbearing potential must have a negative pregnancy test at the Screening and Treatment Initiation Visits 12. Females of reproductive potential* and sexually active must agree to use Food and Drug Administration (FDA) approved methods of birth control for the duration of the study including the open-label and follow-up portions of the study. These include hormonal contraceptives, intrauterine device, double barrier contraception (i.e., condom plus diaphragm), or male partner with documented vasectomy. *Menopause is defined as at least 12 consecutive months without menses; if in question, a follicle stimulating hormone of ≥ 25 U/mL must be documented. Hysterectomy, bilateral oophorectomy, or bilateral tubal ligation must be documented, as applicable; if documented, women with these conditions are not required to use additional contraception. 13. Males who are sexually active must agree to use an acceptable method of birth control (e.g. barrier methods with vaginal spermicide, surgical sterilization or surgically sterilized partner), or have a female partner practicing an approved birth control method for females as described in Inclusion Criterion #12. 14. Willing and able to comply with all clinic visits and study-related procedures 15. Able to understand and complete study-related questionnaires
Exclusion Criteria	<p>Individuals who meet any of these criteria are not eligible for enrollment as study participants:</p> <ol style="list-style-type: none"> 1. Inability or unwillingness of an individual to give written informed consent or comply with study protocol 2. Known systemic hypersensitivity to any of the excipients of the dupilumab or placebo study products

	<ol style="list-style-type: none"> 3. Known or suspected immunosuppression, including history of invasive opportunistic infections (e.g., tuberculosis, histoplasmosis, listeriosis, coccidioidomycosis, pneumocystosis, aspergillosis) despite infection resolution, or otherwise recurrent immune-compromised status, as judged by the investigator 4. Known history of human immunodeficiency virus (HIV) infection 5. Ocular disorder that in the opinion of the investigator could adversely affect the individual's risk for study participation. Examples include, but are not limited to, individuals with a history of or active case of herpes keratitis; Sjogren's Syndrome, Keratoconjunctivitis Sicca or Dry Eye Syndrome that require daily use of supplemental lubrication; or individuals with ocular conditions that require the regular use of ocular corticosteroids or cyclosporine. 6. Parasitic infection, except for vaginal trichomoniasis, within 12 months of the Treatment Initiation Visit, or high risk for contracting parasitic infections (e.g., living in or traveling to endemic areas) 7. Presence of skin comorbidities that may interfere with study assessments 8. History of malignancy within 5 years before the Treatment Initiation Visit except completely treated in situ carcinoma of the cervix, and completely treated and resolved non-metastatic squamous or basal cell carcinoma of the skin or melanoma in situ 9. History of non-malignant lymphoproliferative disorders 10. History of alcohol or drug abuse within 2 years before the Screening Visit 11. Severe concomitant illness(es) that, in the investigator's judgment, would adversely affect the individual's participation in the study. Examples include, but are not limited to, individuals with short life expectancy, uncontrolled diabetes (HbA1c $\geq 9\%$), cardiovascular conditions (e.g., stage III or IV cardiac failure according to the New York Heart Association classification), severe renal conditions (e.g., individuals on dialysis), hepato-biliary conditions (e.g., Child-Pugh class B or C), neurological conditions (e.g., demyelinating diseases), active major autoimmune diseases (e.g., lupus, inflammatory bowel disease, rheumatoid arthritis, etc.), eosinophilic conditions (e.g. eosinophilic granulomatosis with polyangiitis), other
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	<p>severe endocrinological, gastrointestinal, metabolic, pulmonary, or lymphatic diseases.</p> <ol style="list-style-type: none"> 12. Any other medical or psychological condition including relevant laboratory abnormalities at screening that, in the opinion of the investigator, suggests a new and/or insufficiently understood disease, may present an unreasonable risk to the study participant as a result of his/her participation in this clinical trial, may make individual's participation unreliable, or may interfere with study assessments. This includes hypersensitivity to local anesthetics (e.g., lidocaine or Novocain), bleeding disorders, treatment with anticoagulants or other conditions that make the biopsy procedure inadvisable. 13. Planned major surgical procedure during the screening period or study treatment (i.e. Screening through Day 112) 14. Member of the investigational team or his/her immediate family 15. Pregnant or breast-feeding women, or women planning to become pregnant or breastfeed during the study including the open-label and follow up portions of the study 16. Individuals unwilling to use adequate birth control, if of reproductive potential and sexually active. Adequate birth control is defined as agreement to consistently practice an approved method of contraception for the duration of the study including the open-label and follow up portions of the study. Refer to Inclusion criteria #12 and #13. 17. History of keloid formation 18. History of serious life-threatening reaction to tape or adhesives 19. Prior treatment with dupilumab 20. Individuals with asthma who have required use of a systemic corticosteroid within 3 months prior to the Treatment Initiation Visit or who require a dose greater than 880 mcg/day of fluticasone propionate or equivalent inhaled corticosteroid to maintain asthma control 21. Treatment with biologics as follows: <ul style="list-style-type: none"> • Any cell-depleting agents, including but not limited to rituximab, within 6 months before the Treatment Initiation Visit, or until lymphocyte and CD 19+ lymphocyte count returns to normal, whichever is longer • Infliximab, adalimumab, golimumab, certolizumab pegol, abatacept, etanercept, anakinra within 16
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	<p>weeks before the Treatment Initiation Visit for any indication</p> <ul style="list-style-type: none"> • Other biologics within 5 half-lives (if known) or 16 weeks before the Treatment Initiation Visit, whichever is longer <p>22. Treatment with a live (attenuated) vaccine within 12 weeks before the Treatment Initiation Visit or planning to receive a live vaccine during the study (through Day 182)</p> <p>23. Use of an investigational drug within 8 weeks or within 5 half-lives (if known), whichever is longer, before the Treatment Initiation Visit</p> <p>24. Chronic or acute infection requiring treatment with systemic antibiotics, antivirals, antiparasitics, antiprotozoals, or antifungals within 4 weeks before the Treatment Initiation Visit, or superficial skin infections within 1 week before the Treatment Initiation Visit</p> <p>25. The following treatments within 4 weeks before the Treatment Initiation Visit, or any condition that, in the opinion of the investigator, will likely require such treatment(s) during the screening period and study treatment (i.e., Screening through Day 112):</p> <ul style="list-style-type: none"> • Systemic corticosteroids • Immunosuppressive/immunomodulating drugs (e.g., cyclosporine, mycophenolate-mofetil, IFN-γ, Janus kinase inhibitors, azathioprine, or methotrexate) <p>26. Use of phototherapy (such as narrow band ultraviolet B [NBUVB], ultraviolet B [UVB], ultraviolet A1 [UVA1], psoralen + UVA [PUVA]) or a tanning booth/parlor within 4 weeks of the Treatment Initiation Visit</p> <p>27. Treatment with bleach bath within 3 weeks before the Treatment Initiation Visit</p> <p>28. Use of a chlorinated hot tub within 3 weeks before the Treatment Initiation Visit</p> <p>29. Treatment with topical corticosteroids, phosphodiesterase inhibitors (crisaborole), or calcineurin inhibitors (tacrolimus or pimecrolimus) within 1 week before the Treatment Initiation Visit. Low potency topical corticosteroids may be used just on the face up until 48 hours prior to the Treatment Initiation Visit.</p> <p>30. Initiation of treatment of AD with prescription moisturizers or moisturizers containing ceramide, hyaluronic acid, urea, or filaggrin during the screening period (participants may</p>
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	<p>continue using stable doses of such moisturizers if initiated before the Screening Visit)</p> <p>31. Planned or anticipated use of any prohibited medications or procedures during the screening period and study treatment (i.e., Screening through Day 112). Refer to Section 7.3 for additional details.</p>
Study Stopping Rules	<p>Study enrollment will be suspended pending Division of Allergy, Immunology, and Transplantation (DAIT) National Institute of Allergy and Infectious Diseases (NIAID) and NIAID Allergy and Asthma Data Safety Monitoring Board (DSMB) expedited review of all pertinent data if any of the following occur:</p> <ol style="list-style-type: none">1. 1 death, or life-threatening adverse event, that is possibly related to dupilumab2. A grade 3 or higher adverse event that is possibly related to dupilumab in two or more participants

Table of Contents

Glossary of Abbreviations	18
1. Background and Rationale	22
1.1. Background and Scientific Rationale.....	22
1.1.1. Skin Colonization with <i>S. aureus</i>	22
1.1.2. Skin Barrier Dysfunction.....	23
1.2. Rationale for Selection of Investigational Product or Intervention	24
1.2.1 Dupilumab – Rationale.....	24
1.2.2 Efficacy and Safety	24
1.2.3 Dupilumab Mechanistic Studies.....	24
1.2.4 Selection of Dupilumab over Alternative Systemic Biologics Targeting Th2 Cytokines.....	24
1.3. Preclinical Experience	24
1.3.1. In Vitro Studies.....	25
1.3.2. Animal Pharmacology and Safety Studies.....	25
1.3.2.1. Pharmacology.....	25
1.3.2.2. Absorption, Bioavailability, Distribution, Metabolism and Elimination/Excretion	26
1.3.2.3. Toxicology	26
1.3.2.4. Reproductive and Developmental Toxicology	26
1.3.2.5. Carcinogenicity.....	26
1.4. Clinical Studies	27
1.4.1. Clinical Pharmacology	27
1.4.1.1. Mechanism of Action	27
1.4.1.2. Pharmacodynamics	27
1.4.1.3. Pharmacokinetics.....	27
1.4.1.3.1. Absorption.....	27
1.4.1.3.2. Distribution	27
1.4.1.3.3. Elimination	28
1.4.1.3.4. Dose Linearity.....	28
1.4.1.3.5. Weight.....	28
1.4.1.3.6. Age	28
1.4.1.3.7. Immunogenicity	28
1.4.1.3.8. Specific Populations	28
1.4.1.3.9. Drug Interaction Studies	28
1.4.2. Clinical Trials Efficacy	29

1.4.2.1.	Clinical Response at Week 16 (Trials 1, 2, and 3).....	29
1.4.3.	Clinical Trials Safety.....	31
1.4.3.1.	Weeks 0 to 16 (Trials 1 to 4)	31
1.4.3.2.	Safety through Week 52 (Trial 3)	32
1.4.3.3.	Specific Adverse Reactions.....	33
1.4.3.3.1.	Conjunctivitis	33
1.4.3.3.2.	Eczema Herpeticum and Herpes Zoster	33
1.4.3.3.3.	Hypersensitivity Reactions	33
1.4.3.3.4.	Eosinophils	33
1.4.3.3.5.	Cardiovascular	33
2.	Study Hypothesis/Objectives	34
2.1.	Hypothesis.....	34
2.2.	Primary Objective.....	34
2.3.	Secondary Objective(s)	34
2.4.	Exploratory Objective(s).....	34
3.	Study Design.....	34
3.1.	Description of Study Design	34
3.2.	Primary Endpoint(s)/Outcome(s).....	36
3.3.	Secondary Endpoint(s)/Outcome(s).....	36
3.4.	Exploratory Endpoint(s)/Outcome(s).....	36
3.5.	Stratification, Randomization, and Blinding/Masking	37
3.6.	Procedure for Unblinding/Unmasking	37
4.	Selection of Participants and Clinical Sites/Laboratories.....	38
4.1.	Rationale for Study Population	38
4.2.	Inclusion Criteria	38
4.3.	Exclusion Criteria.....	39
4.4.	Selection of Clinical Sites/Labs.....	41
5.	Known and Potential Risks and Benefits to Participants	41
5.1.	Risks of Investigational Product or Intervention as cited in the Package Insert.....	41
5.2.	Risks of Investigational Product or Intervention Cited in Medical Literature.....	42
5.3.	Risks of Other Protocol Specified Medications.....	42
5.4.	Risks of Study Procedures	42
5.4.1	Risks Associated with Stopping the Use of Protocol Prohibited Medications/Procedures	42
5.4.2.	Risks Associated with Physical Exam.....	42

5.4.3.	Risks Associated with Health Questionnaires	42
5.4.4.	Risks Associated with Blood Collection.....	42
5.4.5.	Risks Associated with Skin Swab Collection.....	42
5.4.6.	Risks Associated with Skin Barrier TEWL Measurement.....	43
5.4.7.	Risks Associated with Skin Tape Strip Collection	43
5.4.8.	Risks Associated with Skin Biopsies	43
5.5.	Potential Benefits.....	43
6.	Investigational Agent	44
6.1	Investigational Agent	44
6.1.1.	Dupilumab.....	44
6.1.2.	Placebo.....	45
6.2.	Drug Accountability.....	45
6.3.	Assessment of Participant Compliance with Investigational Agent	46
6.4.	Toxicity Prevention and Management.....	46
6.5.	Premature Discontinuation of Investigational Agent	46
7.	Other Medications/Procedures	47
7.1.	Concomitant Medications.....	47
7.1.1.	Protocol-mandated	47
7.1.2.	Other Permitted Concomitant Medications	47
7.2.	Prophylactic Medications.....	47
7.3.	Prohibited Medications/Procedures.....	47
7.4.	Rescue Medications/Procedures	49
8.	Study Procedures	49
8.1.	Recruitment	49
8.2.	Screening (Day -28 to Day -8)	49
8.3.	Treatment Initiation Visit (Day 0, to Occur within 28 Days of Screening)	50
8.4.	Day 3 (\pm 1 Day)	51
8.5.	Day 7 (\pm 2 days).....	52
8.6.	Day 14 (\pm 2 days).....	53
8.7.	Day 21 (\pm 2 days).....	54
8.8.	Day 28 (\pm 2 days).....	55
8.9.	Day 42 (\pm 2 days).....	56
8.10.	Day 56 (\pm 7 days) Home IP Injection	58
8.11.	Day 70 (\pm 7 days) Home IP Injection	58

8.12.	Day 77 (\pm 2 days)	58
8.13.	Day 84 (\pm 7 days) Home IP Injection	59
8.14.	Day 98 (\pm 7 days) Home IP Injection	59
8.15.	Day 112 (\pm 2 days)	59
8.16.	Day 182 Follow-Up Phone Call (\pm 5 days)	60
8.17.	Unscheduled Visits	60
8.18.	Visit Windows.....	61
9.	Mechanistic Assays	61
9.1.	Measurement of <i>S. aureus</i> Abundance.....	61
9.2.	Measurement of <i>S. aureus</i> Superantigens, Toxins, Lipase, and Proteases.....	62
9.3.	Analysis of the Skin Microbiome.....	62
9.3.1.	Microbiome Assessment by 16S rRNA	62
9.3.2.	Metagenomic Analysis of Microbiome	63
9.4.	Functional Assessment of Coagulase Negative Staphylococcal Species (CoNS) Isolates for Antimicrobial Activity 63	
9.5.	Assessment of Lipid Components	63
9.6.	Confocal Imaging of Skin Biopsies.....	64
9.7.	Skin Transcriptomics	64
9.8.	PBMC Immunoprofiling.....	64
9.9.	Analysis of Serum Biomarkers.....	65
9.10.	Genotyping.....	65
10.	Biospecimen Storage.....	65
11.	Criteria for Participant and Study Completion and Premature Study Termination.....	65
11.1.	Participant Completion	65
11.2.	Participant Stopping Rules and Withdrawal Criteria	66
11.3.	Participant Replacement.....	66
11.4.	Follow-up After Early Study Withdrawal.....	66
11.5.	Study Stopping Rules.....	66
12.	Safety Monitoring and Reporting	67
12.1.	Overview	67
12.2.	Definitions	67
12.2.1.	Adverse Event (AE)	67
12.2.2.	Unexpected Adverse Event	68
12.2.3.	Serious Adverse Event (SAE)	68

12.3.	Grading and Attribution of Adverse Events	69
12.3.1.	Grading Criteria	69
12.3.2.	Attribution Definitions	69
12.4.	Collection and Recording of Adverse Events	70
12.4.1.	Collection Period	70
12.4.2.	Collecting Adverse Events	70
12.4.3.	Recording Adverse Events.....	70
12.5.	Reporting of Serious Adverse Events and Adverse Events	71
12.5.1.	Reporting of Serious Adverse Events to DAIT/NIAID	71
12.5.2.	Reporting to Health Authority	71
12.5.3.	Reporting of Adverse Events to the Central IRB	71
12.6.	Pregnancy Reporting.....	71
12.7.	Reporting of Other Safety Information.....	72
12.8.	Review of Safety Information	72
12.8.1.	Medical Monitor Review	72
12.8.2.	DSMB Review	72
13.	Statistical Considerations and Analytical Plan	73
13.1.	Overview	73
13.2.	Endpoints/Outcomes	73
13.3.	Measures to Minimize Bias	73
13.4.	Analysis Plan.....	73
13.4.1.	Analysis Populations	73
13.4.2.	Primary Analysis of Primary Endpoint(s)/Outcome(s)	74
13.4.3.	Supportive Analyses of the Primary Endpoint(s)/Outcome(s).....	74
13.4.4.	Analyses of Secondary and Other Endpoint(s)/Outcome(s)	74
13.4.5.	Analyses of Exploratory Endpoint(s)/Outcome(s).....	75
13.4.6.	Descriptive Analyses	78
13.5.	Interim Analyses.....	78
13.5.1.	Interim Analysis of Efficacy Data.....	78
13.5.2.	Interim Analysis of Safety Data	78
13.5.3.	Futility Analysis	78
13.6.	Statistical Hypotheses.....	78
13.7.	Sample Size Considerations	78
14.	Identification and Access to Source Data	79

14.1.	Source Data	79
14.2.	Access to Source Data	79
15.	Protocol Deviations	79
15.1.	Protocol Deviation Definitions	79
15.2.	Reporting and Managing Protocol Deviations	80
16.	Ethical Considerations and Compliance with Good Clinical Practice.....	80
16.1.	Quality Control and Quality Assurance	80
16.2.	Statement of Compliance	80
16.3.	Informed Consent Process	80
16.4.	Privacy and Confidentiality	81
17.	Publication Policy	81
18.	References	82
	Appendix A: Schedule of Events	88

Glossary of Abbreviations

AAD	American Academy of Dermatology
Ab	Antibody
AD	Atopic Dermatitis
ADA	Anti-drug Antibodies
ADR	Adverse Drug Reaction
ADRN	Atopic Dermatitis Research Network
ADVNI	Atopic Dermatitis Vaccinia Network
AE	Adverse Event
ALT	Alanine Aminotransferase
AST	Aspartate Aminotransferase
CBC	Complete Blood Count
CFR	Code of Federal Regulations
CFU	Colony Forming Units
CLA	Cutaneous Lymphocyte Antigen
CMP	Comprehensive Metabolic Panel
CoNS	Coagulase Negative Staphylococcal Species
CRF	Case Report Form
CTCAE	Common Terminology Criteria for Adverse Events
DAIT	Division of Allergy, Immunology, and Transplantation
DNA	Deoxyribonucleic Acid
DSMB	Data Safety Monitoring Board
EASI	Eczema Area and Severity Index
EC	Ethics Committee
eCRF	Electronic Case Report Form
EDC	Electronic Data Capture
EMA	European Medicines Agency
FDA	Food and Drug Administration
FLG	Filaggrin
GCP	Good Clinical Practice
GD	Gestational Day
HILIC	Hydrophilic Interaction Chromatography
HIV	Human Immunodeficiency Virus

IB	Investigator Brochure
IC	Inhibitory Concentration
ICH	International Conference on Harmonization
IGA	Investigator Global Assessment
IgE	Immunoglobulin E
IL	Interleukin
IND	Investigational New Drug
IRB	Institutional Review Board
ISR	Injection Site Reaction
IUD	Intrauterine Device
IV	Intravenous
kDa	Kilodalton
LC	Langerhans cell
mAb	Monoclonal Antibody
mbQTL	Microbiome Quantitative Trait Loci
mITT	Modified Intent-to-Treat
MOP	Manual of Procedures
NBUVB	Narrow Band Ultraviolet B
NCI	National Cancer Institute
NESS	Nottingham Eczema Severity Score
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institutes of Health
NP	Nasal Polyposis
NRS	Numerical Rating Scale
OHRP	Office for Human Research Protections
OLE	Open-Label Extension
OTU	Operational Taxonomic Unit
<i>P. acnes</i>	<i>Propionibacterium acnes</i>
PARC	Pulmonary and Activation-Regulated Chemokine
PBMC	Peripheral Blood Mononuclear Cell
PBS	Phosphate Buffered Saline
PCA	Pyroglutamic Acid
PCR	Polymerase Chain Reaction

PHI	Personal Health Identifiers
PI	[Site] Principal Investigator
PK	Pharmacokinetics
PP	Per Protocol
PUVA	Psoralen + Ultraviolet A
PVDF	Polyvinylidene Fluoride
QTL	Quantitative Trait Loci
Q2W	Every two weeks
QW	Once weekly
RDBPC	Randomized Double Blind Placebo Controlled
RNA	Ribonucleic Acid
<i>S. aureus</i>	<i>Staphylococcus aureus</i>
SC	Stratum Corneum
SACCC	Statistical and Clinical Coordinating Center
SAE	Serious Adverse Event
SAg	Superantigen
SAR	Suspected Adverse Reaction
SCORAD	SCORing Atopic Dermatitis
SD	Standard deviation
SNP	Single Nucleotide Polymorphism
SOC	System Organ Class
SOP	Standard Operating Procedure
TARC	Thymus and Activation-Regulated Chemokine
TDAR	T-Cell Dependent Antibody Response
TEAE	Treatment-Emergent Adverse Event
TESAE	Treatment-Emergent Serious Adverse Event
TEWL	Transepidermal Water Loss
Th	T Helper
TJ	Tight Junction
TSB	Tryptic Soy Broth
UCA	Urocanic Acid
████	████████████████████
████	████████████████████
UVA1	Ultraviolet A1

UVB	Ultraviolet B
γ c	Common Gamma Chain

1. Background and Rationale

1.1. Background and Scientific Rationale

Atopic dermatitis (AD) is the most common inflammatory skin disease, affecting 15 million Americans with a greater impact on quality of life than any other chronic skin condition (Bin et al, 2016). Despite this, until recently no Food and Drug Administration (FDA)-approved targeted systemic therapies were available for AD. Dupilumab (Dupixent) has recently been approved for the treatment of moderate-to-severe AD, with or without concomitant topical corticosteroids, in adult patients whose disease is not adequately controlled with prescribed topical therapies or for whom such treatments are not advisable. Dupilumab is a fully human monoclonal antibody that inhibits the signaling of T helper (Th)2 cytokines (Interleukin[IL]-4 and IL-13) resulting in rapid and significant clinical improvement in adults with moderate-to-severe AD (Simpson et al, 2016; Thaci et al, 2016; Beck et al, 2014). As such, dupilumab provides an unprecedented opportunity to further understand the relevance of Th2 inflammation on AD pathology, focusing on the microbial, epithelial, and immune compartments. To do this, we have designed a 6 week randomized double blind placebo controlled (RDBPC) trial with high density sampling of these compartments to characterize and quantify changes and their relationship to disease improvements, followed by a 10 week open-label extension of the study. By integrating this multi-scale, high density data, we can model the complex interactions between the host and microbiome with and without Th2 blockade.

A hallmark of AD is the increased type 2 immune response, that is triggered by a wide range of allergens, microbes, such as *Staphylococcus aureus* (*S. aureus*), and even epithelial proteins. This is a systemic disease as demonstrated by Type 2 inflammation which is even present in non-lesional skin (Suarez-Farinas et al, 2011). Furthermore, expansion of skin-homing (CLA+) Th2 cells is observed in the peripheral blood of AD patients with severe disease (Czarnowicki et al, 2015). Lastly, serum markers of Th2 inflammation such as thymus and activation-regulated chemokine (TARC/CCL17) are much higher in AD than in other allergic disorders (Hijnen et al, 2004). Another hallmark of AD is the increased susceptibility to skin colonization with *S. aureus* (Warner et al, 2009). The final feature of the disease is skin barrier dysfunction which is even observed in non-lesional skin. Below we highlight the evidence for the latter two features and summarize the evidence suggesting that they arise as a consequence of Th2 cytokines.

1.1.1. Skin Colonization with *S. aureus*

In the ADRN Registry study, we have found that 43% of AD participants have *S. aureus* that can be cultured from their skin in contrast to < 2% of healthy controls (Ong et al, 2016; Beck et al, 2008; Totté et al, 2016). PCR quantification of *S. aureus* highlights that virtually all AD participants have this pathogen on their skin surface (ADRN unpublished data). This *S. aureus* abundance correlates strongly with skin barrier measurements such as transepidermal water loss (TEWL) ($p=0.004$) (data not shown) suggesting an interaction between microbiome and host. In 2012, the first microbiome analysis on a small number of AD subjects demonstrated an inverse relationship between the diversity of skin microbiota and intraindividual AD disease activity (Kong et al, 2012). Disease severity was associated with a relative increase in *S. aureus* and a decrease in commensals, including *Propionibacterium acnes* (*P. acnes*). Similarly, the ADRN Barrier study observed a reduction in *P. acnes* in AD participants who were culture positive for *S. aureus* (ADRN unpublished data), implicating commensals as critical for the normal skin biogeography (Shu et al, 2013). In support of this idea, the [REDACTED] laboratory [REDACTED] has demonstrated that commensal bacteria, such as *S. epidermidis* and *hominis* produce antimicrobial molecules which restrict *S. aureus* survival (Lai et al, 2009; Lai et al, 2010; Cogen, Yamasaki, Muto et al, 2010; Cogen, Yamasaki, Sanchez et al, 2010; Nakatsuji et al, 2017). Unfortunately, quantifying the relative (e.g. 16s RNA microbiome [REDACTED]) or even absolute amount of different bacterial species (e.g. qPCR of both pathogens and commensals – [REDACTED]) is a first step in understanding AD skin

biography, but it does not tell us how these bacteria affect the host. For example, *S. aureus* strains differ in their secretion of virulence factors, which are important for amplifying inflammation and inducing class switching of B cells. Whether these toxins and virulence factors are produced can be suggested by the study of the bacterial genome (e.g. metagenome – [REDACTED]) and direct toxin/virulence measurements ([REDACTED]). Metagenomic sequencing catalogs all the genes from uncultivated microbes directly from the site of interest. Metagenomics will be complemented by the direct measurement of *S. aureus* superantigens (SAGs), toxins, lipase, and proteases as has been developed in the laboratory of [REDACTED] (Vu et al, 2015). In summary, type 2 immunity has been implicated as the etiology of the skin dysbiosis observed in AD patients. In this protocol, we will employ complementary assays to give us an unprecedented qualitative and quantitative view of the skin bacterial flora to test whether Th2 blockade reduces *S. aureus* relative or absolute abundance, alters levels of and antimicrobial activity of commensals, and/or changes the toxigenic potential of *S. aureus* (as well as other bacteria).

1.1.2. Skin Barrier Dysfunction

The skin has two barrier structures, the stratum corneum (SC) and tight junctions (TJ). The SC is widely accepted to be dysfunctional based on: 1) alterations in SC lipids (Van Smeden and Bouwstra, 2016), 2) acquired or genetic defects in structural proteins such as filaggrin (FLG), loricrin, and other epidermal differentiation complex genes or 3) simply by scratching in response to intense itch (Matsui et al, 2015). Interestingly, in ADRN studies, *FLG* null mutations were not strongly implicated in European American AD participants' susceptibility to *S. aureus* suggesting that other barrier defects and/or Th2 inflammation may be the explanations (Yoshida et al, 2015; Rafaels et al, 2016). Preliminary lipidomic analysis of skin tape strips from ADRN AD participants identified a number of abnormalities (Berdyshev et al, 2017) which are largely recapitulated in keratinocytes stimulated with IL-4/IL-13, implicating type 2 immunity as the cause (Goleva et al, 2017). TJs are ring-like structures found below the SC, which function as a "gate" for paracellular transport, and are composed of transmembrane proteins including claudin family members (De Benedetto et al, 2011). ADRN studies demonstrated a remarkable defect in epidermal TJ integrity in AD non-lesional skin (De Benedetto et al, 2011). It is presumed that a leaky SC and TJ barrier promotes greater immunologic responsiveness to allergens and is responsible for the physiological abnormalities noted on the skin surface (e.g. reduced SC hydration, increased pH, enhanced TEWL). A number of groups have demonstrated that Th2 (but not Th17) cytokines adversely affect SC and TJ barrier functions (De Benedetto et al, 2015; Howell et al, 2009; Honzke et al, 2016; Yokouchi et al, 2015). These observations support our hypothesis that Th2 blockade, achieved with dupilumab treatment, will repair skin barrier function. In this protocol, we will monitor dupilumab effects on skin barrier by: 1) measuring physiological changes (TEWL – before and after tape stripping [e.g. SC integrity assay]), 2) lipidomics, 3) transcriptomics of epithelial barrier genes and 4) confocal images of TJ structures and their relationship to Langerhans Cells (LC) dendrites.

In summary, it is clear that there is a dynamic interplay between the immune system, epithelial barrier function, and the skin bacterial flora, and these interactions are likely driving some, if not all, of the clinical features that we associate with AD. We have proposed a study with a targeted systemic therapy that inhibits Th2 signaling to monitor the change in barrier function and skin flora in relationship to improvements in skin appearance and symptomatology to begin to address the relative importance of these different functions in disease severity.

1.2. Rationale for Selection of Investigational Product or Intervention

1.2.1 Dupilumab – Rationale

Dupilumab is a fully human mAb directed against the IL-4 receptor alpha subunit (IL-4R α), common to the Type 1 and Type 2 receptors that mediate most of the biological functions of IL-4 and IL-13. It is the first biologic therapy FDA-approved for the treatment of adults with moderate-to-severe AD.

1.2.2 Efficacy and Safety

Dupilumab treatment results in marked and rapid clinical improvement, with improvements observed in Eczema Area and Severity Index (EASI) scores by 2 weeks with greater than 80% improvement by 6 weeks (Simpson et al, 2016; Thaci et al, 2016; Beck et al, 2014). Dramatic and early reductions in pruritus, as measured by the numerical rating scale (NRS) and the 5-D pruritus scale, were also observed. The drug was well-tolerated with few serious adverse events attributable to dupilumab administration in AD trials (Simpson et al, 2016; Thaci et al, 2016; Beck et al, 2014).

1.2.3 Dupilumab Mechanistic Studies

Several exploratory endpoints have been collected as part of Regeneron-sponsored studies and include serum biomarkers (indicative of type 2 immunity), skin *S. aureus* abundance (microbial DNA), PBMC immunoprofiling and skin transcriptome. A pharmacodynamic response, as indicated by reduction in the Th2 biomarker, TARC/CCL17, could be observed as early as 1 week (Beck et al, 2014). None of the other readouts were performed before 4 weeks of dupilumab treatment. Skin transcriptome studies demonstrated that after 4 weeks of dupilumab treatment the lesional skin begins to approximate the non-lesional skin transcriptome (Hamilton et al, 2014). In a different study, a significant reduction in *S. aureus* abundance was observed in lesional skin after 4 weeks ($p < 0.05$), which was even more significant by 16 weeks of dupilumab treatment ($p < 0.01$) (Guttman-Yassky et al, 2016). Non-lesional swabs showed the same trend. This is consistent with observations made only in the Phase 2 studies where skin infections were reported more commonly in the placebo-treated group (24% vs 6%, respectively) (Beck et al, 2014). Our protocol differs from the aforementioned studies as it will focus on frequent assessments of all relevant compartments (skin microbiome, barrier, and immunoprofile) with an emphasis on early time points (≤ 6 weeks); we will improve the sample processing \pm assay methodology, will be the first to address effects on skin barrier function, will significantly enhance the depth and breadth of microbial analyses, and most of these measures will be performed in all study participants. This will provide a rich data-driven network to model and predict disease improvement [REDACTED] We will also take advantage of an open-label extension (providing 16 weeks and 10 weeks of continuous dupilumab treatment for participants randomized on entry to either active or placebo groups, respectively) to add to the disease severity modeling with a focus on intraindividual variation during dupilumab treatment.

1.2.4 Selection of Dupilumab over Alternative Systemic Biologics Targeting Th2 Cytokines

Only two other systemic biologics targeting Th2 cytokines are in clinical development, namely lebrikizumab and tralokinumab. Both are directed against IL-13 and are at least a year behind dupilumab in clinical development. For this reason and because we hypothesize that blocking both IL-4 and IL-13 may be more effective, we have chosen dupilumab as the drug of choice to test the hypotheses outlined in this protocol.

1.3. Preclinical Experience

The following comes from the dupilumab Investigator's Brochure [REDACTED]

[REDACTED]

1.3.1. In Vitro Studies

1.3.1.1. [REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]

1.3.1.2. [REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]

1.3.1.3. [REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]

1.3.1.4. [REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]

1.3.2. [REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]

1.3.2.2.

[REDACTED]

1.3.2.3.

[REDACTED]

1.3.2.4.

[REDACTED]

[REDACTED]

1.3.2.5.

[REDACTED]

1.4. Clinical Studies

The following comes from the dupilumab package insert [REDACTED]

1.4.1. Clinical Pharmacology

1.4.1.1. Mechanism of Action

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

1.4.1.2. Pharmacodynamics

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

1.4.1.3. Pharmacokinetics

The pharmacokinetics of dupilumab [REDACTED]

1.4.1.3.1. Absorption

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

1.4.1.3.2. Distribution

[REDACTED]

1.4.1.3.3. Elimination

[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]

1.4.1.3.4. Dose Linearity

[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]

1.4.1.3.5. Weight

[REDACTED]

1.4.1.3.6. Age

[REDACTED]

1.4.1.3.7. Immunogenicity

[REDACTED]
[REDACTED]
[REDACTED]

1.4.1.3.8. Specific Populations

[REDACTED]

[REDACTED]
[REDACTED]
[REDACTED]

[REDACTED]

[REDACTED]
[REDACTED]

1.4.1.3.9. Drug Interaction Studies

[REDACTED]
[REDACTED]
[REDACTED]

[REDACTED]

[REDACTED]
[REDACTED]
[REDACTED]
[REDACTED]

[REDACTED]

1.4.2. Clinical Trials Efficacy

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

1.4.2.1. Clinical Response at Week 16 (Trials 1, 2, and 3)

[REDACTED]

[illegible]

29 Aug 2019

Table 1.4.2.1b [REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

1.4.3. Clinical Trials Safety

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

1.4.3.1. Weeks 0 to 16 (Trials 1 to 4)

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

1.4.3.3. Specific Adverse Reactions**1.4.3.3.1.**

[REDACTED]

1.4.3.3.2.

[REDACTED]

1.4.3.3.3.

[REDACTED]

1.4.3.3.4.

[REDACTED]

1.4.3.3.5.

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

2. Study Hypothesis/Objectives

2.1. Hypothesis

We hypothesize that dupilumab treatment will significantly alter the skin bacterial flora; specifically reducing abundance of *S. aureus* (lesionally and non-lesionally) as well as normalizing skin barrier function and these changes will be reflected in disease improvement.

2.2. Primary Objective

To assess the effect of dupilumab on *S. aureus* abundance on lesional skin as measured by microbial DNA

2.3. Secondary Objective(s)

1. To assess the effect of dupilumab on *S. aureus* abundance on non-lesional skin as measured by microbial DNA
2. To assess the effect of dupilumab on epidermal barrier function in lesional and non-lesional skin
3. To assess the effect of dupilumab on disease severity

2.4. Exploratory Objective(s)

1. To assess the effect of dupilumab on the microbiome and metagenome in lesional and non-lesional skin
2. To assess the effect of dupilumab on the skin transcriptome in lesional and non-lesional skin [REDACTED] participants only)
3. To assess the effect of dupilumab on epidermal lipids in lesional and non-lesional skin
4. To assess the effect of dupilumab on the expression of *S. aureus* superantigens, toxins, lipase, and proteases on lesional and non-lesional skin
5. To assess the effect of dupilumab on non-lesional skin barrier structure and Langerhans cells (LC) by confocal imaging ([REDACTED] participants only)
6. To assess the effect of dupilumab on the function of the skin microbiome (i.e. ability of Coagulase-negative staphylococci isolates [CoNS] to kill *S. aureus*) in lesional and non-lesional skin
7. To assess the effect of dupilumab on PBMC immunoprofiles
8. To assess the effect of dupilumab on serum biomarkers (e.g. Th2 biomarkers)
9. To explore the association between genetic variation and (clinical and mechanistic) responsiveness to dupilumab
10. To identify networks/pathways associated with responsiveness to dupilumab, quantified by disease severity, symptoms, and barrier function

3. Study Design

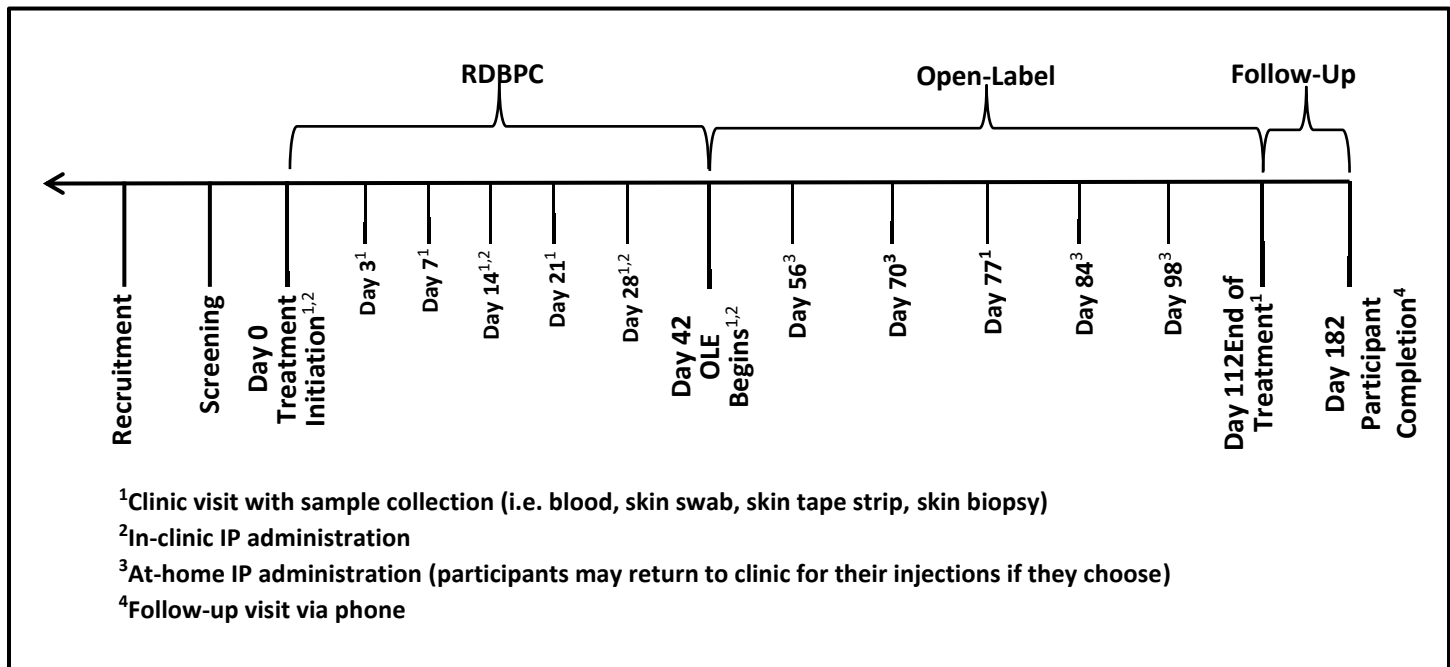
3.1. Description of Study Design

This is a multi-center, RDBPC study investigating the effect of 6 weeks of dupilumab treatment on quantitative and qualitative measures of cutaneous microbial community structure, skin barrier biology, skin transcriptome, and circulating T cell profiles in adults with moderate-to-severe AD, followed by a 10 week open-label extension of the study for all participants. We have chosen a 6 week period for the RDBPC portion of the study based on findings from Phase 2 and 3 studies that demonstrated that greater than 80% of the maximum improvement seen in EASI score was observed by that time point (Thaci et al, 2016; Simpson et al, 2016). Approximately 99 (66 active: 33 placebo) adult AD participants, 18 to 75 years of age, will be enrolled for this study.

During the Screening Visit, participants will provide informed consent for study participation. Consented participants will then be assessed for study eligibility through the collection of medical history, including medication use and history of medical procedures, a physical exam, assessments of AD severity, a Comprehensive Metabolic Panel (CMP), a Complete Blood Count (CBC) with differential, and a pregnancy test for female participants of child-bearing potential.

Participants who are eligible will return to clinic for their Treatment Initiation Visit (Day 0) and will be randomized 2:1 active to placebo. Participants will receive three doses of dupilumab or placebo based on their randomization assignment. The first dose (600 mg loading dose or placebo) will be administered on Day 0 and the second and third doses (300 mg or placebo) on Day 14 and Day 28, respectively. Participants will also return to clinic on Days 3, 7, and 21 during the double-blind portion of the study. Participants will begin the OLE at Day 42 and will receive dupilumab (600 mg loading dose [two 300 mg injections] for those initially randomized to the placebo group and a 300 mg dose plus placebo injection for those initially randomized to the dupilumab group). Participants or their caregiver will administer one 300 mg dose of dupilumab at home on Day 56, Day 70, Day 84, and Day 98. If participants are unable or unwilling to self-administer or have a caregiver administer injections at home, they will be provided with the option of returning to the study clinic to have a staff member administer the injection. Participants will return to clinic on Days 77 and 112 during the OLE portion of the study. During all visits (Day 0-Day 112), AEs, concomitant medications, and medical history will be assessed and physical exams including assessment of AD severity will be performed. Blood, urine, skin swabs, skin tape strips, and skin biopsies, as applicable, will be collected, and barrier assessments will be performed per the Schedule of Events (Appendix A). Samples will be collected prior to dupilumab or placebo administration on Days 0, 14, 28, and 42. After Day 112, a follow-up call (Day 182) will be made to assess for pregnancy, current medications, and AEs.

If concerns arise between regularly scheduled visits, participants will be instructed to contact study personnel and may be asked to return to the study site for an "Unscheduled Visit." Participants may be asked to return for Unscheduled Visits, as needed for the duration of the study, to provide additional blood, skin swabs, skin tape strips, or skin biopsies, as applicable, for further mechanistic and functional studies, or if samples are lost or destroyed, or if insufficient yields were obtained at a previous study visit.

Figure 3.1 Study Design Schematic**3.2. Primary Endpoint(s)/Outcome(s)**

S. aureus abundance as measured by microbial DNA (*femA* qPCR) on lesional skin at Day 28

3.3. Secondary Endpoint(s)/Outcome(s)

1. *S. aureus* abundance as measured by microbial DNA (*femA* qPCR) on lesional skin at Days 0, 3, 7, 14, 21, 42, 77 and 112
2. *S. aureus* abundance as measured by microbial DNA (*femA* qPCR) on non-lesional skin at Days 0, 3, 7, 14, 21, 28, 42, 77 and 112
3. Basal (prior to tape stripping) transepidermal water loss (TEWL) of non-lesional and lesional skin at Days 0, 3, 7, 14, 21, 28, 42, 77 and 112
4. TEWL area under the curve of non-lesional skin at Days 0, 7, 14, 21, 28, 42, 77 and 112. TEWL will be assessed prior to tape stripping and repeated after 5, 10, and 15 tape strips.
5. Change in TEWL per every 5 tape strips (i.e. slope) on non-lesional skin at Days 0, 7, 14, 21, 28, 42, 77 and 112
6. Eczema Area and Severity Index [EASI], Investigator Global Assessment [IGA], and SCORing Atopic Dermatitis [SCORAD] at Days 0, 3, 7, 14, 21, 28, 42, 77 and 112
7. Pruritus numerical rating scale [NRS] at Days 0, 3, 7, 14, 21, 28, 42, 77 and 112

3.4. Exploratory Endpoint(s)/Outcome(s)

1. Composition of bacterial taxa in lesional and non-lesional skin at Days 0, 3, 7, 14, 21, 28, 42, 77 and 112
2. Abundance of bacterial taxa in lesional and non-lesional skin at Days 0, 3, 7, 14, 21, 28, 42, 77 and 112
3. Gene expression in the skin transcriptome in non-lesional skin at Days 0 and 7, and in lesional skin at Days 0, 7, and 21
4. Lipid profiles of non-lesional skin at Days 0, 7, 14, 21, 28, 42, 77 and 112 and of lesional skin at Days 0, 14, 28, and 112
5. Expression of *S. aureus* superantigens, toxins, lipase, and proteases on lesional and non-lesional skin at Days 0, 3, 7, 14, 21, 28, 42, 77 and 112

6. Confocal imaging of tight junctions (TJs) and relationship to LCs in the epidermis from non-lesional skin at Days 0, 7 and 21
7. Percent of coagulase-negative staphylococci [CoNS] isolates that kill *S. aureus* on lesional and non-lesional skin at Days 0, 3, 7, 14, 21, 28, 42, 77 and 112
8. PBMC immunoprofiling at Days 0, 14 and 28
9. Levels of serum biomarkers (e.g. Th2 biomarkers and ADA) at Days 0, 7, 14, 21, 28, 42, 77 and 112
10. The presence of single nucleotide polymorphisms (SNPs)

3.5. Stratification, Randomization, and Blinding/Masking

Randomization will be performed by the clinical sites using a validated interactive web response system (IWRS) created by the Statistical and Clinical Coordinating Center (SACCC) that automates the random assignment of treatment groups to study ID numbers. The randomization scheme will be reviewed by a statistician at the SACCC prior to implementation. Participants will be randomized using a 2:1 ratio of active (dupilumab) and control (placebo) participants. A stratified permuted block randomization algorithm will be used to maintain a balance between treatment arms with respect to clinical site and disease severity at Day 0 ($EASI \geq 21.1$ or < 21.1).

Randomization data will be kept strictly confidential, accessible only to authorized persons, until the time of unblinding. When all subject data through Day 112 is complete, the data files verified, and the protocol deviations determined, the investigational agent codes will be broken and made available for data analysis.

The clinical site's research pharmacy will dispense the study drug as per the randomization schedule provided by the SACCC. The unblinded pharmacist will confirm the expiration date, the dose, and randomization assignment. The injections will be administered by the blinded study physician or other qualified medical professional during the RDBPC portion of the study. Clinical research staff assessing clinical endpoints and participants will be blinded to treatment assignments for the RDBPC portion through the end of the trial. In order to maintain the blind for the RDBPC portion of the study, at the initiation of the OLE portion of the study, participants who were randomized to dupilumab for the RDBPC portion will receive one 300 mg injection of dupilumab and one placebo injection, while participants who were randomized to placebo will receive two 300 mg injections of dupilumab. Clinical research staff and participants will be aware of the treatment being received during the OLE portion of the study, as all participants will receive active treatment; neither the clinical research staff nor the participants will be told the original treatment assignment during the OLE portion of the study. Laboratory staff processing and analyzing samples will be blinded to participant treatment assignments during the RDBPC portion of the trial until all samples required for analysis are processed and raw data is available.

3.6. Procedure for Unblinding/Unmasking

If a clinically significant event occurs and knowledge of the treatment assignment is required, the study treatment may be unblinded. Unblinding must be approved by the Division of Allergy, Immunology, and Transplantation (DAIT)/National Institute of Allergy and Infectious Diseases (NIAID) Medical Monitor unless an immediate life-threatening condition has developed and the DAIT/NIAID Medical Monitor is not accessible. In the event of an emergency, the investigator or designated qualified individual may obtain the participant's blinded treatment assignment via the IWRS. Upon performance of a blind break, the IWRS will send out a blinded notification to alert Statistical and Clinical Coordinating Center and NIAID staff. In the case of an accidental or emergency unblinding outside of IWRS, the site investigator will notify the DAIT/NIAID Medical Monitor and the SACCC team of the unblinding event by the next business day, following the unblinding. The unblinding will also be reported to the NIAID Asthma and Allergy Data and Safety Monitoring Board (DSMB).

A full account of the event will be recorded, including the date and time of the unblinding, the reason for the decision to unblind, the extent of the unblinding, the name of the individual who made the decision to unblind, and the names of the DAIT/NIAID Medical Monitor and others who were notified. The reasons for unblinding of a participant's treatment will be included in the final study report.

Unblinding the study will require written approval from NIAID. No formal interim analysis is planned for this study. In order to conduct analyses related to the scientific objectives, unblinding will occur after all participants have completed the Day 112 Visit and associated laboratory assays have been completed. Refer to Section 13.5.1 for additional details.

4. Selection of Participants and Clinical Sites/Laboratories

4.1. Rationale for Study Population

The purpose of this study is to understand the effect that Th2 blockade has on well-described pathophysiological features of AD (e.g. barrier, epidermal activation, dysbiosis, epidermal lipids, etc). This study will be limited to male and female adult participants, ages 18-75 years, with moderate-to-severe AD. Most human studies performed to date with dupilumab have been restricted to adults (≥ 18 years of age) and for this reason and the fact that we will be asking participants to undergo skin biopsies, which are not part of routine health care for a pediatric population, we have limited our studies to adults. We have put an upper age limit on our study population because skin barrier function/structure deteriorates in elderly individuals, and we want to minimize the effect this confounding variable would have on our study interpretations.

4.2. Inclusion Criteria

Individuals who meet all of the following criteria are eligible for enrollment as study participants:

1. Must be able to understand and provide informed consent
2. Male or female, 18-75 years inclusive at the Screening Visit
3. Chronic AD, (according to the ADRN Standard Diagnostic Criteria), that has been present for at least 3 years before the Screening Visit for individuals aged 18-65 years old OR for at least 5 years before the Screening Visit for individuals aged 66-75 years old. Individuals aged 66-75 years of age must have documentation of a skin biopsy to rule out cutaneous T-cell lymphoma and other non-AD conditions present in the medical record.
4. EASI score ≥ 12 at the Screening Visit and ≥ 16 at the Treatment Initiation Visit
5. Investigator Global Assessment (IGA) score ≥ 3 (on the 0-4 IGA scale) at the Screening and Treatment Initiation Visits
6. $\geq 10\%$ body surface area of AD involvement at the Screening and Treatment Initiation Visits
7. Must have active lesions (minimum of 3 of at least 4×4 cm² each on the upper or lower extremities or trunk, excluding the palms of the hands, soles of the feet, buttock, navel, axilla, suprapubic area, and neck) at the Screening and Treatment Initiation Visits
8. Documented recent history (within 6 months before the Screening Visit) of inadequate response to outpatient treatment with topical corticosteroids of medium to high potency (\pm topical calcineurin inhibitors as appropriate), or for whom topical treatments are otherwise inadvisable (e.g., because of important side effects or safety risks). Acceptable documentation includes contemporaneous chart notes that record prescription of topical corticosteroids and/or topical calcineurin inhibitors, investigator documentation based on communication with patient's treating physician, or medical history provided by the patient.
9. Must agree to apply a stable dose of a topical emollient (moisturizer) at least twice daily for at least 7 days before the Treatment Initiation Visit, and must confirm application at the Treatment Initiation Visit

10. Individuals with asthma must adhere to asthma controller medication(s) for the duration of the study including the open-label and follow-up portions
11. Females of childbearing potential must have a negative pregnancy test at the Screening and Treatment Initiation Visits
12. Females of reproductive potential* and sexually active must agree to use FDA approved methods of birth control for the duration of the study including the open-label and follow-up portions of the study. These include hormonal contraceptives, intrauterine device, double barrier contraception (i.e., condom plus diaphragm), or male partner with documented vasectomy.
*Menopause is defined as at least 12 consecutive months without menses; if in question, a follicle stimulating hormone of ≥ 25 U/mL must be documented. Hysterectomy, bilateral oophorectomy, or bilateral tubal ligation must be documented, as applicable; if documented, women with these conditions are not required to use additional contraception.
13. Males who are sexually active must agree to use an acceptable method of birth control (e.g. barrier methods with vaginal spermicide, surgical sterilization or surgically sterilized partner), or have a female partner practicing an approved birth control method for females as described in Inclusion Criterion #12.
14. Willing and able to comply with all clinic visits and study-related procedures
15. Able to understand and complete study-related questionnaires

4.3. Exclusion Criteria

Individuals who meet any of these criteria are not eligible for enrollment as study participants:

1. Inability or unwillingness of an individual to give written informed consent or comply with study protocol
2. Known systemic hypersensitivity to any of the excipients of the dupilumab or placebo study products
3. Known or suspected immunosuppression, including history of invasive opportunistic infections (e.g., tuberculosis, histoplasmosis, listeriosis, coccidioidomycosis, pneumocystosis, aspergillosis) despite infection resolution, or otherwise recurrent immune-compromised status, as judged by the investigator
4. Known history of human immunodeficiency virus (HIV) infection
5. Ocular disorder that in the opinion of the investigator could adversely affect the individual's risk for study participation. Examples include, but are not limited to, individuals with a history of or active case of herpes keratitis; Sjogren's Syndrome, Keratoconjunctivitis Sicca or Dry Eye Syndrome requiring daily use of supplemental lubrication; or individuals with ocular conditions that require the regular use of ocular corticosteroids or cyclosporine.
6. Parasitic infection, except for vaginal trichomoniasis, within 12 months of the Treatment Initiation Visit, or high risk for contracting parasitic infections (e.g., living in or traveling to endemic areas)
7. Presence of skin comorbidities that may interfere with study assessments
8. History of malignancy within 5 years before the Treatment Initiation Visit except completely treated in situ carcinoma of the cervix, and completely treated and resolved non-metastatic squamous or basal cell carcinoma of the skin or melanoma in situ
9. History of non-malignant lymphoproliferative disorders
10. History of alcohol or drug abuse within 2 years before the Screening Visit
11. Severe concomitant illness(es) that, in the investigator's judgment, would adversely affect the individual's participation in the study. Examples include, but are not limited to, individuals with short life expectancy, uncontrolled diabetes (HbA1c $\geq 9\%$), cardiovascular conditions (e.g., stage III or IV cardiac failure according to the New York Heart Association classification), severe renal conditions (e.g., individuals on dialysis), hepato-biliary conditions (e.g., Child-Pugh class B or C), neurological conditions (e.g., demyelinating

diseases), active major autoimmune diseases (e.g., lupus, inflammatory bowel disease, rheumatoid arthritis, etc.), eosinophilic conditions (e.g. eosinophilic granulomatosis with polyangiitis), other severe endocrinological, gastrointestinal, metabolic, pulmonary, or lymphatic diseases.

12. Any other medical or psychological condition including relevant laboratory abnormalities at screening that, in the opinion of the investigator, suggests a new and/or insufficiently understood disease, may present an unreasonable risk to the study participant as a result of his/her participation in this clinical trial, may make individual's participation unreliable, or may interfere with study assessments. This includes hypersensitivity to local anesthetics (e.g., lidocaine or Novocain), bleeding disorders, treatment with anticoagulants or other conditions that make the biopsy procedure inadvisable.
13. Planned major surgical procedure during the screening period or study treatment (i.e. Screening through Day 112)
14. Member of the investigational team or his/her immediate family
15. Pregnant or breast-feeding women, or women planning to become pregnant or breastfeed during the study including the open-label and follow up portions of the study
16. Individuals unwilling to use adequate birth control, if of reproductive potential and sexually active. Adequate birth control is defined as agreement to consistently practice an approved method of contraception for the duration of the study including the open-label and follow up portions of the study. Refer to Inclusion criteria #12 and #13.
17. History of keloid formation
18. History of serious life-threatening reaction to tape or adhesives
19. Prior treatment with dupilumab
20. Individuals with asthma who have required use of a systemic corticosteroid within 3 months prior to the Treatment Initiation Visit or who require a dose greater than 880 mcg/day of fluticasone propionate or equivalent inhaled corticosteroid to maintain asthma control
21. Treatment with biologics as follows:
 - Any cell-depleting agents, including but not limited to rituximab, within 6 months before the Treatment Initiation Visit, or until lymphocyte and CD 19+ lymphocyte count returns to normal, whichever is longer
 - Infliximab, adalimumab, golimumab, certolizumab pegol, abatacept, etanercept, anakinra within 16 weeks before the Treatment Initiation Visit for any indication
 - Other biologics within 5 half-lives (if known) or 16 weeks before the Treatment Initiation Visit, whichever is longer
22. Treatment with a live (attenuated) vaccine within 12 weeks before the Treatment Initiation Visit or planning to receive a live vaccine during the study (through Day 182)
23. Use of an investigational drug within 8 weeks or within 5 half-lives (if known), whichever is longer, before the Treatment Initiation Visit
24. Chronic or acute infection requiring treatment with systemic antibiotics, antivirals, antiparasitics, antiprotozoals, or antifungals within 4 weeks before the Treatment Initiation Visit, or superficial skin infections within 1 week before the Treatment Initiation Visit
25. The following treatments within 4 weeks before the Treatment Initiation Visit, or any condition that, in the opinion of the investigator, will likely require such treatment(s) during the screening period and study treatment (i.e., Screening through Day 112):
 - Systemic corticosteroids

- Immunosuppressive/immunomodulating drugs (e.g., cyclosporine, mycophenolate-mofetil, IFN- γ , Janus kinase inhibitors, azathioprine, or methotrexate)
26. Use of phototherapy (such as narrow band ultraviolet B [NBUVB], ultraviolet B [UVB], ultraviolet A1 [UVA1], psoralen + UVA [PUVA]) or a tanning booth/parlor within 4 weeks of the Treatment Initiation Visit
 27. Treatment with bleach bath within 3 weeks before the Treatment Initiation Visit
 28. Use of a chlorinated hot tub within 3 weeks before the Treatment Initiation Visit
 29. Treatment with topical corticosteroids, phosphodiesterase inhibitors (crisaborole), or calcineurin inhibitors (tacrolimus or pimecrolimus) within 1 week before the Treatment Initiation Visit. Low potency topical corticosteroids may be used just on the face up until 48 hours prior to the Treatment Initiation Visit.
 30. Initiation of treatment of AD with prescription moisturizers or moisturizers containing ceramide, hyaluronic acid, urea, or filaggrin during the screening period (participants may continue using stable doses of such moisturizers if initiated before the Screening Visit)
 31. Planned or anticipated use of any prohibited medications or procedures during the screening period and study treatment (i.e., Screening through Day 112). Refer to Section 7.3 for additional details.

4.4. Selection of Clinical Sites/Labs

This will be a multicenter study ([REDACTED]). All sites will be part of the ADRN. These sites have access to large adult AD populations, experience with clinical trials and performing required study procedures including skin biopsies, skin tape stripping, skin barrier assessments, AD severity scoring, blood/tissue processing, and biomaterials shipping.

The laboratories processing and analyzing the samples for this study are discussed in Section 9. The proposed techniques for successful implementation and completion of the proposed studies have been established in preliminary studies at the respective laboratories.

5. Known and Potential Risks and Benefits to Participants

5.1. Risks of Investigational Product or Intervention as cited in the Package Insert

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

[REDACTED]

5.2. Risks of Investigational Product or Intervention Cited in Medical Literature

Refer to Section 5.1 for reported risks. The medical literature has no additional information.

5.3. Risks of Other Protocol Specified Medications

Not applicable

5.4. Risks of Study Procedures

5.4.1 Risks Associated with Stopping the Use of Protocol Prohibited Medications/Procedures

Risks associated with stopping the use of protocol-prohibited medications/procedures may include worsening of the condition being treated and will be reported as such. In an effort to minimize these risks, participants with severe AD or severe asthma who may have difficulty tolerating periods without medication/procedure use will be excluded from participating, per study exclusion criteria.

5.4.2. Risks Associated with Physical Exam

There are no known risks associated with the physical exam.

5.4.3. Risks Associated with Health Questionnaires

There is a possibility that participants may find questions too personal. Participants may refuse to answer any questions that make them feel uncomfortable. There is also a possibility that a participant's answers may be read by others; however, participants' records are carefully protected so this is very unlikely. See Section 16.4 for more information on confidentiality.

5.4.4. Risks Associated with Blood Collection

Risks associated with drawing blood include possible pain when the needle is inserted, as well as bleeding, bruising and/or infection at the puncture site. Some people may experience lightheadedness, nausea, or fainting. A topical anesthetic (e.g. topical lidocaine/prilocaine cream) may be placed on the skin before the blood draw to reduce the pain of the stick. Side effects from this cream (mainly skin rash) may occur. National Institutes of Health (NIH) guidelines for blood collection (amount and frequency based on age) will be followed.

5.4.5. Risks Associated with Skin Swab Collection

There are no significant risks associated with skin swab collection.

5.4.6 Risks Associated with Skin Barrier TEWL Measurement

There are no known risks associated with this non-invasive skin measurement.

5.4.7. Risks Associated with Skin Tape Strip Collection

Risks associated with skin tape stripping, theoretically, include the rare possibility of an allergic reaction to the tape or a skin infection. Since the tape is removed immediately after application, the risk of an allergic reaction is extremely low. However, in previous and ongoing studies involving tape stripping, it has been noted that a very mild erythema may develop immediately after a series of tape strips on one localized area of skin, presumably due to the mild mechanical disturbance. The erythema is expected to resolve within 12 hours without sequelae. The risk of skin infection is extremely low since only superficial skin layers are removed. A bandage will be applied to the area of tape stripping to reduce the small likelihood of an infection. Some people may experience lightheadedness, nausea, or fainting. Possible bleeding and/or bruising may also occur at the area. Participants with a history of serious life-threatening reaction to tape or adhesives will be excluded from participating, per study exclusion criteria.

5.4.8. Risks Associated with Skin Biopsies

Risks of skin biopsy include pain and the possibility of an adverse reaction consisting of local swelling, bleeding, infection, and scar formation. The pain associated with injection of a local anesthetic, such as lidocaine, is mild and transitory. Allergic reactions to lidocaine are extremely rare and occur in less than 1 in 10,000 individuals who receive lidocaine. Reactions can be mild to life-threatening. Allergic reactions could result in hives, shortness of breath, an asthma attack, or anaphylactic shock. Anaphylactic shock is the most severe form of an allergic reaction. It could lead to complete failure of the heart and circulation and could result in more health problems, or death. However, anaphylactic shock is extremely rare and occurs in less than 30 in 100,000 individuals who receive lidocaine. Symptoms occur within minutes to 2 hours, but in rare instances may occur up to 4 hours later. Individuals with asthma, eczema, or hay fever are at greater relative risk of experiencing anaphylaxis. Occasionally, participants may experience swelling at the injection site. Significant bleeding from the biopsy site(s) is rare and infrequent. Infection of a biopsy site is unusual, but may occur. A small scar may result at the biopsy site. Participants who receive skin biopsies will be given wound care instructions. Participants who have a lidocaine or Novocain allergy or those with a history of keloid formation will be excluded from participating, per study exclusion criteria.

5.5. Potential Benefits

There may or may not be any direct benefits for the participants who elect to enroll in this study. Participants assigned to the dupilumab treatment arm during the double-blind portion of the study will receive a total of 16 weeks of treatment (6 weeks double-blind and 10 weeks open-label), and those assigned to the placebo arm will receive a total of 10 weeks of dupilumab treatment during the OLE portion of the study. One potential benefit for participants is that their AD may improve and their itch may be reduced while on dupilumab; however, there is no guarantee that the product will help the participant's condition and 33% of participants will receive placebo during the first 6 weeks of the study and not the active study drug. The participant's skin condition may even get worse by withholding his/her previous/regular AD treatment, or the participant could be in the placebo group and not receive active medication for the first 6 weeks of the study, in which case his/her AD may also flare.

Although the results of this study may be of commercial value, it will be explained to participants that they will not have ownership of these results, and will not benefit financially from participation in this study, other than the nominal reimbursements provided for completing study procedures and visits. The potential benefit to society is significant if it

[REDACTED]

6.1.2. Placebo

6.1.2.1. Formulation, Packaging, and Labeling

[REDACTED]

[REDACTED]

[REDACTED]

6.1.2.2. Dosage, Preparation, and Administration

[REDACTED]

[REDACTED]

[REDACTED]

6.2. Drug Accountability

Under Title 21 of the Code of Federal Regulations (21CFR §312.62), the investigator will maintain adequate records of the disposition of the investigational products, including the date and quantity of the drug/placebo received, to whom the drug/placebo was dispensed (participant-by-participant accounting), and a detailed accounting of any drug/placebo accidentally or deliberately destroyed. Details of investigational product dispensation to each participating clinical site will be maintained at Eminent Services, Inc.

Records for receipt, storage, use, and disposition will be maintained by the clinical site. A drug/placebo-dispensing log will be kept current for each participant. This log will contain the identification number of each participant and the date and quantity of drug/placebo dispensed.

All records regarding the disposition of the investigational products will be available for inspection. At the termination of the study, all unused product will be returned to Eminent for destruction. For more information on handling of the investigational product, please refer to the manual of procedures (MOP).

6.3. Assessment of Participant Compliance with Investigational Agent

Study product will be administered in the clinic through Day 42 by site personnel and thus is an observed compliance for the RDBPC portion of the study. Reminder calls will be provided for self-administered doses on Days 56, 70, 84, and 98, as outlined in the study MOP. Compliance for the OLE will be based on participant self-report. Compliance will be determined by the percentage of injections received.

6.4. Toxicity Prevention and Management

Dose modification for an individual participant is not allowed. Study drug may be prematurely discontinued as delineated in Section 6.5. If absolutely necessary (e.g., for treatment of intolerable AD symptoms or super-infection), a prohibited medication or procedure (as defined in Section 7.3) may be allowed at the discretion of the investigator (See Section 7.4).

6.5. Premature Discontinuation of Investigational Agent

Investigational products may be prematurely discontinued for any participant for any of the following reasons:

- Anaphylactic reaction to study drug injection
- Diagnosis of a malignancy during study, excluding carcinoma in situ of the cervix, or squamous or basal cell carcinoma of the skin
- Evidence of pregnancy
- Any infection that:
 - Requires parenteral treatment with an antibiotic, antifungal, antiviral, antiparasitic, or antiprotozoal agent
 - Requires oral treatment with such agents for longer than 2 weeks
 - Is opportunistic, such as tuberculosis and other infections whose nature or course may suggest an immune-compromised status
- Severe worsening of AD, which in the opinion of the investigator requires stopping of the study medication
- Evidence of severe and prolonged ISRs to study medication
- Evidence of non-compliance to study protocol that in the opinion of the investigator requires discontinuation of study medication
- Any serious AE that is possibly or definitely related to the investigational product

Investigational product may also be prematurely discontinued for any participant if the investigator believes that continuing use of the investigational product is no longer in the best interest of the participant.

If a participant is prematurely discontinued from investigational product, but not withdrawn from the study, the participant will be asked and encouraged to complete any remaining study visits according to the Schedule of Events (Appendix A).

7. Other Medications/Procedures

7.1. Concomitant Medications

Any treatment (including nutritional supplements) or procedure administered from the time of consent to the end of study visits is considered a concomitant medication/procedure. This includes permitted medications ongoing at the time of consent.

7.1.1. Protocol-mandated

Participants are required to apply a stable dose of a topical emollient at least twice daily for at least 7 days before the Treatment Initiation Visit. Participants are prohibited from initiating treatment with prescription moisturizers or moisturizers containing ceramide, hyaluronic acid, urea, or filaggrin, but may continue using stable doses of such moisturizers if initiated before the Screening Visit.

7.1.2. Other Permitted Concomitant Medications

Other than the prohibited medications and procedures listed in Section 7.3, treatment with concomitant medications and procedures is permitted during the study. This includes treatment with contraceptives, nasal and inhaled corticosteroids, and oral antihistamines.

7.2. Prophylactic Medications

Female participants of child-bearing potential must use an effective method of contraception (e.g. total abstinence, oral contraceptives, IUDs, barrier method with spermicide, surgical sterilization or surgically sterilized partner, Depo-Provera, Norplant, NuvaRing, or hormonal implants) for the duration of study participation including the open-label and follow up portions of the study.

Pregnancy tests will be performed on female participants of child-bearing potential at the Screening, Treatment Initiation (Day 0), Day 14, Day 28, Day 42, and Day 77 Visits. These participants will also be asked during their other Clinic Visits and Follow-Up Phone Call as to whether they have tested positive to a pregnancy test since their last study visit.

Male participants will be asked if a partner has tested positive to a pregnancy test at each Clinic Visit and Follow-Up Phone Call.

All reported pregnancies, in female participants and the partners of male participants, will be followed as described in Section 12.6.

7.3. Prohibited Medications/Procedures

Treatment with the following concomitant medications and procedures is prohibited through Day 112.

- Medications used for the treatment of AD or super-infection (these are considered rescue medications):
 - Topical calcineurin inhibitors (tacrolimus or pimecrolimus)
 - Topical phosphodiesterase inhibitors (crisaborole)

- Topical corticosteroids
 - Topical antibiotics
 - Initiation of prescription moisturizers or moisturizers containing ceramide, hyaluronic acid, urea, or filaggrin. Participants may continue using stable doses of such moisturizers if initiated before the Screening Visit.
 - Systemic treatment for AD with an immunosuppressive/immunomodulating agent (including, but not limited to, systemic corticosteroids, cyclosporine, mycophenolate-mofetil, azathioprine, methotrexate, IFN- γ , or other biologics)
- Treatment with biologics including, but not limited to, the following:
 - Any cell-depleting agents (e.g. rituximab)
 - Infliximab, adalimumab, golimumab, certolizumab pegol, abatacept, etanercept, or anakinra
- Medications used for the treatment of asthma:
 - Systemic corticosteroids
 - Inhaled corticosteroids at a dose greater than 880 mcg/day of fluticasone propionate or equivalent
- Systemic antibiotics, antivirals, antiparasitics, antiprotozoals, or antifungals
- Allergen immunotherapy (AIT), unless the participant has been on maintenance therapy for at least six months prior to the Treatment Initiation Visit. Participants on maintenance therapy for at least six months can continue AIT as long as no changes are made to dosing through Day 112.
- Treatment with an investigational drug
- Major elective surgical procedures
- Procedures used for the treatment of AD (these are considered rescue procedures):
 - Phototherapy (such as NBUVB, UVB, UVA1, or PUVA)
 - Bleach baths
- Use of a tanning booth/parlor

In addition, participants will be asked to abstain from live (attenuated) vaccinations through the follow-up portion of the study (Day 182). If a participant requires a vaccination prior to 12 weeks after discontinuing treatment with dupilumab, titers should be checked post-vaccination. Live (attenuated) vaccinations include but are not limited to the following:

- BCG
- Chickenpox (Varicella)
- FluMist-Influenza
- Intranasal influenza
- Measles (Rubeola)
- Measles-mumps-rubella (MMR) combination
- Measles-mumps-rubella-varicella (MMRV) combination
- Mumps
- Oral polio (Sabin)
- Oral typhoid
- Rotavirus
- Rubella
- Smallpox (Vaccinia)
- Varicella Zoster (shingles)

- Yellow fever

7.4. Rescue Medications/Procedures

If absolutely necessary (e.g. for treatment of intolerable AD symptoms or super-infection), a prohibited medication or procedure (as defined in Section 7.3) may be allowed at the discretion of the investigator. Participants who receive rescue treatment with a prohibited medication or procedure through Day 112 will be asked to continue with study treatment and procedures per the Schedule of Events (Appendix A) unless they meet Participant Stopping Rules (Section 11.2).

In the event of an infection or allergic reaction, best clinical practices will be followed for participant treatment. Infections will be treated with anti-bacterials based on the antibiotic sensitivity of the infection. Allergic reactions to the topical anesthetic, lidocaine, tape strips, or study drug will be treated with antihistamine and/or epinephrine depending on the severity of the reaction.

8. Study Procedures

A summary of complete study procedures is included in the Schedule of Events (Appendix A).

8.1. Recruitment

Potential participants will be recruited using standardized questionnaires that collect contact information and medical history related to inclusion and exclusion criteria. Participants may be recruited by phone or in person. Once recruitment has been initiated, the participant will be assigned a unique participant identification (ID) number. Those who have no obvious characteristics making them ineligible for the study and who are interested in participating will be invited to clinic to complete the Screening Visit.

8.2. Screening (Day -28 to Day -8)

The purpose of the Screening Visit is to confirm eligibility to continue in the study. The study will be explained in lay terms to each potential participant. During the visit, written informed consent will be obtained from the participant prior to performing any study procedures.

The following procedures, assessments, and laboratory measures will be conducted to determine participant eligibility:

- Collection of demographics and contact information
- Medical history and physical examination by a study physician or other qualified medical professional. The physical examination will include assessments of AD severity (EASI, IGA, SCORAD, and Pruritis NRS).
- Confirm pregnancy status, including performing a urine pregnancy test for female participants of child-bearing potential
- Assessment of current medications
- Vital signs, including temperature, blood pressure, heart rate, respiratory rate; and growth parameters, including height and weight
- Blood collection; approximately 2 mL for a CBC with differential
- Blood collection; approximately 4 mL for a CMP
- Assessment of AEs

Participants who do not meet inclusion and exclusion criteria due to assessment of their current medications/procedures will be asked whether they would be willing to come off their medications/procedures for a

washout period. Participants who do not wish to washout the prohibited medications/procedures will be identified as screen failures and will not continue in the study.

Participants will be advised of situations that would put them at high risk for contracting a parasitic infection. Participants with asthma will be reminded that they should continue to take their asthma medications and will be counseled that they should only change their asthma medication in consultation with a physician.

At the conclusion of the Screening Visit, participants who are eligible will be asked to return to clinic for their Treatment Initiation Visit. Participants will be instructed to apply a stable dose of emollient at least twice a day for at least 7 days prior to the Treatment Initiation Visit. Participants will be provided instructions regarding the use of emollients, including a reminder to not apply emollient 24 hours prior to the Treatment Initiation Visit. Participants will be asked to refrain from the use of prohibited medications/procedures as described in Section 7.3. Participants will be given an instructional hand card with restrictions on bathing/showering and use of chlorinated pools and hot tubs. Sexually active participants of child-bearing potential will be instructed to use an acceptable method of contraception as defined in Section 4.3.

8.3. Treatment Initiation Visit (Day 0, to Occur within 28 Days of Screening)

The purpose of the Treatment Initiation Visit (Day 0) is to initiate study treatment and conduct clinical and AE assessments. The following procedures, assessments, and laboratory measures will be conducted at the initial treatment visit:

- Interim medical history and physical examination, including assessments of AD severity (EASI, IGA, NESS, SCORAD, and Pruritus NRS)
- Confirm pregnancy status of female participants of child-bearing potential and partners of male participants, including performing a urine pregnancy test for female participants of child-bearing potential
- Assessment of concomitant medications
- Vital signs, including temperature, blood pressure, heart rate, and respiratory rate
- Randomization
- Blood collection; approximately 20 mL for assessment of serum biomarkers (e.g., Th2 biomarkers and ADA)
- Blood collection; approximately 40 mL for PBMC immunoprofiling
- Blood collection; approximately 2 mL for DNA isolation if not previously collected for another ADRN or Atopic Dermatitis Vaccinia Network (ADVNI) study
- Photographs will be taken of the measured lesional and non-lesional sites for collection of skin swabs, tape strips, and biopsies. These photographs will be used to identify the areas at subsequent visits.
- Skin swab collection; 3 lesional and 3 non-lesional swabs to be used to determine *S. aureus* abundance; *S. aureus* superantigen, toxin, lipase, protease quantitation; metagenomics, microbiome 16S rRNA analysis; and skin microbiome functional assessment.
- Skin barrier assessments (TEWL basal and after 5, 10, and 15 tape strips)
- Skin tape strip collection; 1 set of 15 strips from non-lesional skin for lipidomics. These strips will be obtained as part of the TEWL barrier assessment.
- Skin tape strip collection; 1 set of 5 strips from lesional skin for lipidomics
- Skin biopsy collection; 1 x 3 mm non-lesional skin biopsy for confocal imaging [REDACTED]
- Skin biopsy collection; 1 x 2.5 mm lesional biopsy and 1 x 2.5 mm non-lesional biopsy for skin transcriptomics [REDACTED]

- IP administration (loading dose); two subcutaneous injections of dupilumab (300 mg) or two subcutaneous injections of placebo based on treatment assignment at two different injection sites (injections will be administered in any of the four abdominal quadrants, thighs, or upper arms).
- Assessment of AEs

If a participant has not refrained from the use of prohibited medications/procedures (as described in Section 7.3) or followed instructions for bathing/showering, use of chlorinated pools and hot tubs, and use of emollients prior to this visit, the visit will be rescheduled.

Clinic staff will educate participants and/or caregivers on administering study product beginning at the Day 0 Visit. Participants or their caregivers will be expected to complete supervised study product administration at a minimum of one visit if the participant or caregiver intends to administer injections at home during the OLE portion of the study. The investigator or other qualified staff must feel confident that the participant or their caregiver can administer study product per the study protocol before they will be provided with study product for home administration.

At the conclusion of this visit, participants will be reminded to refrain from the use of prohibited medications/procedures as described in Section 7.3. Participants will be given instructions regarding restrictions on bathing/showering, use of chlorinated pools and hot tubs, and use of emollients, including instructions not to apply emollients to sampling areas 24 hours prior to study visits. Sexually active participants of child-bearing potential will be instructed to use an acceptable method of contraception as described in Section 4.3. Participants will be advised of situations that would put them at high risk for contracting a parasitic infection.

Participants with asthma will be reminded that they should continue to take their asthma medications and will be counseled that they should only change their asthma medication in consultation with a physician. A letter will be provided to alert the physician managing their asthma to contact the study physician if changes to the asthma medication regimen will be made.

Participants will be provided with instructions on when to contact the research clinic should they experience any adverse events, including new onset of eye symptoms. This card will include symptoms of an allergic/anaphylactic reaction. Contact information for the study physician and the 24 hour on call physician will also be provided. The physician receiving the call will assess if the participant will need to be seen in clinic or if any further treatment is necessary. Alternatively, the participants will be instructed to take an emergency contact hand card with them should they seek medical care at another facility. The emergency contact hand card will include contact information for the research site and will state that the participant is currently in a clinical trial involving the administration of dupilumab, a fully human monoclonal antibody against the IL-4 receptor.

8.4. Day 3 (\pm 1 Day)

The purpose of the Day 3 Visit is to conduct clinical and AE assessments. The following procedures, assessments, and laboratory measures will be conducted at the Day 3 Visit:

- Interim medical history and physical examination, including assessments of AD severity (EASI, IGA, SCORAD, and Pruritus NRS)
- Confirm pregnancy status of female participants of child-bearing potential and of partners of male participants
- Assessment of concomitant medications
- Vital signs, including temperature, blood pressure, heart rate, and respiratory rate

- Photographs will be taken of the measured lesional and non-lesional sites for skin swab collection. These sites should be the same areas that were selected and photographed during the Treatment Initiation Visit. The areas swabbed should be the same size as the areas swabbed during the Treatment Initiation Visit, even if the lesion is smaller. Even if the lesion has cleared, the sample(s) should be labeled as “lesional.”
- Skin swab collection; 3 lesional and 3 non-lesional swabs to be used to determine *S. aureus* abundance; *S. aureus* superantigen, toxin, lipase, and protease quantitation; metagenomics, microbiome 16S rRNA analysis; and skin microbiome functional assessment.
- Skin barrier assessments (TEWL basal)
- Assessment of AEs

Assessments and sample collection will be completed for all participants regardless of whether they used prohibited medications/procedures or met sampling criteria. Protocol deviations will be recorded, as applicable.

At the conclusion of this visit, participants will be reminded to refrain from the use of prohibited medications/procedures as described in Section 7.3. Participants will be given instructions regarding restrictions on bathing/showering, use of chlorinated pools and hot tubs, and use of emollients, including instructions not to apply emollients to sampling areas 24 hours prior to study visits. Sexually active participants of child-bearing potential will be instructed to use an acceptable method of contraception as described in Section 4.3. Participants will be advised of situations that would put them at high risk for contracting a parasitic infection. Participants with asthma will be reminded that they should continue to take their asthma medications and will be counseled that they should only change their asthma medication in consultation with a physician. Participants will be reminded to contact the research clinic should they experience any adverse events, including new onset of eye symptoms.

8.5. Day 7 (\pm 2 days)

The purpose of the Day 7 Visit is to conduct clinical and AE assessments. The following procedures, assessments, and laboratory measures will be conducted at the Day 7 Visit:

- Interim medical history and physical examination, including assessments of AD severity (EASI, IGA, SCORAD, and Pruritus NRS)
- Confirm pregnancy status of female participants of child-bearing potential and partners of male participants
- Assessment of concomitant medications
- Vital signs, including temperature, blood pressure, heart rate, and respiratory rate
- Blood collection; approximately 20 mL for serum biomarkers (e.g., Th2 biomarkers and ADA)
- Photographs will be taken of the measured lesional and non-lesional sites for collection of skin swabs, tape strips, and biopsies. These sites should be the same areas that were selected and photographed during the Treatment Initiation Visit. The areas swabbed should be the same size as the areas swabbed during the Treatment Initiation Visit, even if the lesion is smaller. Even if the lesion has cleared, the sample(s) should be labeled as “lesional”.
- Skin swab collection; 3 lesional and 3 non-lesional swabs to be used to determine *S. aureus* abundance; *S. aureus* superantigen, toxin, lipase, and protease quantitation; metagenomics, microbiome 16S rRNA analysis; and skin microbiome functional assessment.
- Skin barrier assessments (TEWL basal and after 5, 10, and 15 tape strips)
- Skin tape strip collection; 1 set of 15 strips from non-lesional skin for lipidomics. These strips will be obtained as part of the TEWL barrier assessment.
- Skin biopsy collection; 1x 3 mm non-lesional skin biopsy for confocal imaging [REDACTED]

- Skin biopsy collection; 1 x 2.5 mm lesional biopsy and 1 x 2.5 mm non-lesional biopsy for skin transcriptomics [REDACTED]
- Assessment of AEs

Assessments and sample collection will be completed for all participants regardless of whether they used prohibited medications/procedures or met sampling criteria. Protocol deviations will be recorded, as applicable.

At the conclusion of this visit, participants will be reminded to refrain from the use of prohibited medications/procedures as described in Section 7.3. Participants will be given instructions regarding restrictions on bathing/showering, use of chlorinated pools and hot tubs, and use of emollients, including instructions not to apply emollients to sampling areas 24 hours prior to study visits. Sexually active participants of child-bearing potential will be instructed to use an acceptable method of contraception as described in Section 4.3. Participants will be advised of situations that would put them at high risk for contracting a parasitic infection. Participants with asthma will be reminded that they should continue to take their asthma medications and will be counseled that they should only change their asthma medication in consultation with a physician. Participants will be reminded to contact the research clinic should they experience any adverse events, including new onset of eye symptoms.

8.6. Day 14 (± 2 days)

The purpose of the Day 14 Visit is to administer study treatment and conduct clinical and AE assessments. The following procedures, assessments, and laboratory measures will be conducted at the Day 14 Visit:

- Interim medical history and physical examination, including assessments of AD severity (EASI, IGA, SCORAD, and Pruritus NRS)
- Confirm pregnancy status of females of child-bearing potential and partners of male participants, including performing a urine pregnancy test for female participants of child-bearing potential
- Assessment of concomitant medications
- Vital signs, including temperature, blood pressure, heart rate, and respiratory rate
- Blood collection; approximately 20 mL for serum biomarkers (e.g., Th2 biomarkers and ADA)
- Blood collection; approximately 40 mL for PBMC immunoprofiling
- Photographs will be taken of the measured lesional and non-lesional sites for collection of skin swabs and tape strips. These sites should be the same areas that were selected and photographed during the Treatment Initiation Visit. The areas swabbed should be the same size as the areas swabbed during the Treatment Initiation Visit, even if the lesion is smaller. Even if the lesion has cleared, the sample(s) should be labeled as “lesional”.
- Skin swab collection; 3 lesional and 3 non-lesional swabs to be used to determine *S. aureus* abundance, *S. aureus* superantigen toxin, lipase, and protease quantitation; metagenomics, microbiome 16S rRNA analysis and skin microbiome functional assessment.
- Skin barrier assessments (TEWL basal and after 5, 10, and 15 tape strips)
- Skin tape strip collection; 1 set of 15 strips from non-lesional skin for lipidomics. These strips will be obtained as part of the TEWL barrier assessment.
- Skin tape strip collection; 1 set of 5 strips from lesional skin for lipidomics
- IP administration; one 300 mg dupilumab subcutaneous injection or one placebo injection in the abdomen, thigh, or upper arm. The selected injection site should be different from the sites used for the loading dose two weeks earlier.
- Assessment of AEs

Assessments and sample collection will be completed for all participants regardless of whether they used prohibited medications/procedures or met sampling criteria. Protocol deviations will be recorded, as applicable.

Clinic staff will educate participants and/or caregivers on administering study product at the Day 14 Visit. The participant or their caregiver may complete study product administration under the supervision of the investigator or other qualified staff at the Day 14 Visit. Participants or their caregivers will be expected to complete supervised study product administration at a minimum of one visit if the participant or caregiver intends to administer injections at home during the OLE portion of the study. The investigator or other qualified staff must feel confident that participants or their caregiver can administer study product per the study protocol before they will be provided with study product for home administration.

At the conclusion of this visit, participants will be reminded to refrain from the use of prohibited medications/procedures as described in Section 7.3. Participants will be given instructions regarding restrictions on bathing/showering, use of chlorinated pools and hot tubs, and use of emollients, including instructions not to apply emollients to sampling areas 24 hours prior to study visits. Sexually active participants of child-bearing potential will be instructed to use an acceptable method of contraception as described in Section 4.3. Participants will be advised of situations that would put them at high risk for contracting a parasitic infection. Participants with asthma will be reminded that they should continue to take their asthma medications and will be counseled that they should only change their asthma medication in consultation with a physician.

Participants will be provided with instructions on when to contact the research clinic should they experience any adverse events, including new onset of eye symptoms. This card will include symptoms of an allergic/anaphylactic reaction. Contact information for the study physician and the 24 hour on call physician will also be provided. The physician receiving the call will assess if the participant will need to be seen in clinic or if any further treatment is necessary. Alternatively, the participants will be instructed to take an emergency contact hand card with them should they seek medical care at another facility. The emergency contact hand card will include contact information for the research site and will state that the participant is currently in a clinical trial involving the administration of dupilumab, a fully human monoclonal antibody against the IL-4 receptor.

8.7. Day 21 (± 2 days)

The purpose of the Day 21 Visit is to conduct clinical and AE assessments. The following procedures, assessments, and laboratory measures will be conducted at the Day 21 Visit:

- Interim medical history and physical examination, including assessments of AD severity (EASI, IGA, SCORAD, and Pruritus NRS)
- Confirm pregnancy status of female participants of child-bearing potential and partners of male participants
- Assessment of concomitant medications
- Vital signs, including temperature, blood pressure, heart rate, and respiratory rate
- Blood collection; approximately 20 mL for serum biomarkers (e.g., Th2 biomarkers and ADA)
- Photographs will be taken of the measured lesional and non-lesional sites for collection of skin swabs, tape strips, and biopsies. These sites should be the same areas that were selected and photographed during the Treatment Initiation Visit. The areas swabbed should be the same size as the areas swabbed during the Treatment Initiation Visit, even if the lesion is smaller. Even if the lesion has cleared, the sample(s) should be labeled as “lesional”.

- Skin swab collection; 3 lesional and 3 non-lesional swabs to be used to determine *S. aureus* abundance, *S. aureus* superantigen, toxin, lipase, and protease quantitation; metagenomics, microbiome 16S rRNA analysis and skin microbiome functional assessment.
- Skin barrier assessments (TEWL basal and after 5, 10, and 15 tape strips)
- Skin tape strip collection; 1 set of 15 strips from non-lesional skin for lipidomics. These strips will be obtained as part of the TEWL barrier assessment.
- Skin biopsy collection; 1 x 3 mm non-lesional skin biopsy for confocal imaging [REDACTED]
- Skin biopsy collection; 1 x 2.5 mm lesional biopsy for skin transcriptomics [REDACTED]
- Assessment of AEs

Assessments and sample collection will be completed for all participants regardless of whether they used prohibited medications/procedures or met sampling criteria. Protocol deviations will be recorded, as applicable.

At the conclusion of this visit, participants will be reminded to refrain from the use of prohibited medications/procedures as described in Section 7.3. Participants will be given instructions regarding restrictions on bathing/showering, use of chlorinated pools and hot tubs, and use of emollients, including instructions not to apply emollients to sampling areas 24 hours prior to study visits. Sexually active participants of child-bearing potential will be instructed to use an acceptable method of contraception as described in Section 4.3. Participants will be advised of situations that would put them at high risk for contracting a parasitic infection. Participants with asthma will be reminded that they should continue to take their asthma medications and will be counseled that they should only change their asthma medication in consultation with a physician.

Participants will be reminded to contact the research clinic should they experience any adverse events, including new onset of eye symptoms.

8.8. Day 28 (± 2 days)

The purpose of the Day 28 Visit is to administer study treatment and conduct clinical and AE assessments. The following procedures, assessments, and laboratory measures will be conducted at the Day 28 Visit:

- Interim medical history and physical examination, including assessments of AD severity (EASI, IGA, SCORAD, and Pruritus NRS)
- Confirm pregnancy status of females of child-bearing potential and partners of male participants, including performing a urine pregnancy test for female participants of child-bearing potential
- Assessment of concomitant medications
- Vital signs, including temperature, blood pressure, heart rate, and respiratory rate
- Blood collection; approximately 20 mL for serum biomarkers (e.g., Th2 biomarkers and ADA)
- Blood collection; approximately 40 mL for PBMC immunoprofiling
- Photographs will be taken of the measured lesional and non-lesional sites for collection of skin swabs and tape strips. These sites should be the same areas that were selected and photographed during the Treatment Initiation Visit. The areas swabbed should be the same size as the areas swabbed during the Treatment Initiation Visit, even if the lesion is smaller. Even if the lesion has cleared, the sample(s) should be labeled as “lesional.”
- Skin swab collection; 3 lesional and 3 non-lesional swabs to be used to determine *S. aureus* abundance, *S. aureus* superantigen, toxin, lipase, and protease quantitation; metagenomics, microbiome 16S rRNA analysis and skin microbiome functional assessment.
- Skin barrier assessments (TEWL basal and after 5, 10, and 15 tape strips)

- Skin tape strip collection; 1 set of 15 strips from non-lesional skin for lipidomics. These strips will be obtained as part of the TEWL barrier assessment.
- Skin tape strip collection; 1 set of 5 strips from lesional skin for lipidomics
- IP administration; one 300 mg dupilumab subcutaneous injection or one placebo injection in the abdomen, thigh, or upper arm. The selected injection site should be different from the site used for the previous dose.
- Assessment of AEs

Assessments and sample collection will be completed for all participants regardless of whether they used prohibited medications/procedures or met sampling criteria. Protocol deviations will be recorded, as applicable.

Clinic staff will counsel participants and/or caregivers on administering study product at the Day 28 Visit. The participant or their caregiver may complete study product administration under the supervision of the investigator or other qualified staff at the Day 28 Visit. Participants or their caregiver will be expected to complete supervised study product administration at a minimum of one visit if the participant or caregiver intends to administer injections at home during the OLE portion of the study. The investigator or other qualified staff must feel confident that participants or their caregiver can administer study product per the study protocol before they will be provided with study product for home administration.

At the conclusion of this visit, participants will be reminded to refrain from the use of prohibited medications/procedures as described in Section 7.3. Participants will be given instructions regarding restrictions on bathing/showering, use of chlorinated pools and hot tubs, and use of emollients, including instructions not to apply emollients to sampling areas 24 hours prior to study visits. Sexually active participants of child-bearing potential will be instructed to use an acceptable method of contraception as described in Section 4.3. Participants will be advised of situations that would put them at high risk for contracting a parasitic infection. Participants with asthma will be reminded that they should continue to take their asthma medications and will be counseled that they should only change their asthma medication in consultation with a physician.

Participants will be provided with instructions on when to contact the research clinic should they experience any adverse events, including new onset of eye symptoms. This card will include symptoms of an allergic/anaphylactic reaction. Contact information for the study physician and the 24 hour on call physician will also be provided. The physician receiving the call will assess if the participant will need to be seen in clinic or if any further treatment is necessary. Alternatively, the participants will be instructed to take an emergency contact hand card with them should they seek medical care at another facility. The emergency contact hand card will include contact information for the research site and will state that the participant is currently in a clinical trial involving the administration of dupilumab, a fully human monoclonal antibody against the IL-4 receptor.

8.9. Day 42 (\pm 2 days)

The purpose of the Day 42 Visit is to conduct clinical and AE assessments and to begin the OLE. The following procedures, assessments, and laboratory measures will be conducted at the Day 42 Visit:

- Interim medical history and physical examination, including assessments of AD severity (EASI, IGA, SCORAD, and Pruritus NRS)
- Confirm pregnancy status of female participants of child-bearing potential and partners of male participants, including performing a urine pregnancy test for female participants of child-bearing potential
- Assessment of concomitant medications
- Vital signs, including temperature, blood pressure, heart rate, and respiratory rate

- Blood collection; approximately 20 mL for serum biomarkers (e.g., Th2 biomarkers and ADA)
- Photographs will be taken of the measured lesional and non-lesional sites for collection of skin swabs and tape strips. These sites should be the same areas that were selected and photographed during the Treatment Initiation Visit. The areas swabbed should be the same size as the areas swabbed during the Treatment Initiation Visit, even if the lesion is smaller. Even if the lesion has cleared, the sample(s) should be labeled as “lesional”.
- Skin swab collection; 3 lesional and 3 non-lesional swabs to be used to determine *S. aureus* abundance, *S. aureus* superantigen, toxin, lipase, and protease quantitation; metagenomics, microbiome 16S rRNA analysis and skin microbiome functional assessment.
- Skin barrier assessments (TEWL basal and after 5, 10, and 15 tape strips)
- Skin tape strip collection; 1 set of 15 strips from non-lesional skin for lipidomics. These strips will be obtained as part of the TEWL barrier assessment.
- IP administration; In order to maintain the blind, participants who were randomized to dupilumab for the RDBPC portion will receive one 300 mg injection of dupilumab and one placebo injection, while participants who were randomized to placebo will receive two 300 mg injections of dupilumab. The two injections will be administered at different sites in the abdomen, thighs, or upper arms. The selected injection sites should not be the same as the injection site selected for the previous dose.
- Assessment of AEs

Assessments and sample collection will be completed for all participants regardless of whether they used prohibited medications/procedures or met sampling criteria. Protocol deviations will be recorded, as applicable.

Clinic staff will educate participants and/or caregivers on administering study product. The participant or their caregiver must complete study product administration under the supervision of the investigator or other qualified staff at this visit if the participant intends to complete injections at home during the OLE portion of the study. The investigator or other qualified staff must feel confident that participants or their caregiver can administer study product per the study protocol before they will be provided with study product for home administration. Participants who intend to complete injections at home will be provided with two doses of dupilumab for self-administration at home on Days 56 and 70. The participant may alternatively choose to return to the clinic to have their Day 56 and Day 70 doses administered.

At the conclusion of this visit, participants will be reminded to refrain from the use of prohibited medications/procedures as described in Section 7.3. Participants will be given instructions regarding restrictions on bathing/showering, use of chlorinated pools and hot tubs, and use of emollients, including instructions not to apply emollients to sampling areas 24 hours prior to study visits. Sexually active participants of child-bearing potential will be instructed to use an acceptable method of contraception as described in Section 4.3. Participants will be advised of situations that would put them at high risk for contracting a parasitic infection. Participants with asthma will be reminded that they should continue to take their asthma medications and will be counseled that they should only change their asthma medication in consultation with a physician.

Participants will be provided with instructions on when to contact the research clinic should they experience any adverse events, including new onset of eye symptoms. This card will include symptoms of an allergic/anaphylactic reaction. Contact information for the study physician and the 24 hour on call physician will also be provided. The physician receiving the call will assess if the participant will need to be seen in clinic or if any further treatment is necessary. Alternatively, the participants will be instructed to take an emergency contact hand card with them should they seek medical care at another facility. The emergency contact hand card will include contact information for the

research site and will state that the participant is currently in a clinical trial involving the administration of dupilumab, a fully human monoclonal antibody against the IL-4 receptor.

8.10. Day 56 (± 7 days) Home IP Injection

Participant or caretaker will administer 300 mg of dupilumab subcutaneously in the abdomen, thigh, or upper arm. The selected injection site should not be the same as the injection sites selected for the previous dose. If a participant is not comfortable self-administering injections and does not have a caretaker willing to administer injections, the participant may return to the study clinic to have their injection administered. IP injections should occur at minimum 7 days apart. If a participant misses a dose it should be administered within 7 days from the missed dose. If the missed dose is not administered within 7 days, the participant will be instructed to wait until their next scheduled dose based on their original dosing schedule.

8.11. Day 70 (± 7 days) Home IP Injection

Participant or caretaker will administer 300 mg of dupilumab subcutaneously in the abdomen, thigh, or upper arm. The selected injection site should not be the same as the injection site selected for the previous dose. If a participant is not comfortable self-administering injections and does not have a caretaker willing to administer injections, the participant may return to the study clinic to have their injection administered. IP injections should occur at minimum 7 days apart. If a participant misses a dose it should be administered within 7 days from the missed dose. If the missed dose is not administered within 7 days, the participant will be instructed to wait until their next scheduled dose based on their original dosing schedule.

8.12. Day 77 (± 2 days)

The purpose of the Day 77 Visit is to conduct clinical and AE assessments. The following procedures, assessments, and laboratory measures will be conducted at the Day 77 Visit:

- Interim medical history and physical examination, including assessments of AD severity (EASI, IGA, SCORAD, and Pruritus NRS)
- Confirm pregnancy status of female participants of child-bearing potential and partners of male participants, including performing a urine pregnancy test for female participants of child-bearing potential
- Assessment of concomitant medications
- Vital signs, including temperature, blood pressure, heart rate, and respiratory rate
- Blood collection; approximately 20 mL for serum biomarkers (e.g., Th2 biomarkers and ADA)
- Photographs will be taken of the measured lesional and non-lesional sites for collection of skin swabs and tape strips. These sites should be the same areas that were selected and photographed during the Treatment Initiation Visit. The areas swabbed should be the same size as the areas swabbed during the Treatment Initiation Visit, even if the lesion is smaller. Even if the lesion has cleared, the sample(s) should be labeled as “lesional”.
- Skin swab collection; 3 lesional and 3 non-lesional swabs to be used to determine *S. aureus* abundance, *S. aureus* superantigen, toxin, lipase, and protease quantitation; metagenomics, microbiome 16S rRNA analysis and skin microbiome functional assessment.
- Skin barrier assessments (TEWL basal and after 5, 10, and 15 tape strips)
- Skin tape strip collection; 1 set of 15 strips from non-lesional skin for lipidomics. These strips will be obtained as part of the TEWL barrier assessment.
- Assessment of AEs

Assessments and sample collection will be completed for all participants regardless of whether they used prohibited medications/procedures or met sampling criteria. Protocol deviations will be recorded, as applicable.

At the conclusion of this visit, participants who intend to complete injections at home will be provided with two doses of dupilumab for self-administration at home on Days 84 and 98. The participant may alternatively choose to return to the clinic to have their Day 84 and Day 98 doses administered. Participants will be reminded to refrain from the use of prohibited medications/procedures as described in Section 7.3. Participants will be given instructions regarding restrictions on bathing/showering, use of chlorinated pools and hot tubs, and use of emollients, including instructions not to apply emollients to sampling areas 24 hours prior to study visits. Sexually active participants of child-bearing potential will be instructed to use an acceptable method of contraception as described in Section 4.3. Participants will be advised of situations that would put them at high risk for contracting a parasitic infection. Participants with asthma will be reminded that they should continue to take their asthma medications and will be counseled that they should only change their asthma medication in consultation with a physician. Participants will be reminded to contact the research clinic should they experience any adverse events, including new onset of eye symptoms.

8.13. Day 84 (± 7 days) Home IP Injection

Participant or caretaker will administer 300 mg of dupilumab subcutaneously in the abdomen, thigh, or upper arm. The selected injection site should not be the same as the injection site selected for the previous dose. If a participant is not comfortable self-administering injections and does not have a caretaker willing to administer injections, the participant may return to the study clinic to have their injection administered. IP injections should occur at minimum 7 days apart. If a participant misses a dose it should be administered within 7 days from the missed dose. If the missed dose is not administered within 7 days, the participant will be instructed to wait until their next scheduled dose based on their original dosing schedule.

8.14. Day 98 (± 7 days) Home IP Injection

Participant or caretaker will administer 300 mg of dupilumab subcutaneously in the abdomen, thigh, or upper arm. The selected injection site should not be the same as the injection site selected for the previous dose. If a participant is not comfortable self-administering injections and does not have a caretaker willing to administer injections, the participant may return to the study clinic to have their injection administered. IP injections should occur at minimum 7 days apart. If a participant misses a dose it should be administered within 7 days from the missed dose. If the missed dose is not administered within 7 days, the participant will be instructed to wait until their next scheduled dose based on their original dosing schedule.

8.15. Day 112 (± 2 days)

The purpose of the Day 112 Visit is to conduct clinical and AE assessments. The following procedures, assessments, and laboratory measures will be conducted at the Day 112 Visit:

- Interim medical history and physical examination, including assessments of AD severity (EASI, IGA, SCORAD, and Pruritus NRS)
- Confirm pregnancy status of female participants of child-bearing potential and partners of male participants, including performing a urine pregnancy test for female participants of child-bearing potential
- Assessment of concomitant medications
- Vital signs, including temperature, blood pressure, heart rate, and respiratory rate
- Blood collection; approximately 20 mL for serum biomarkers (e.g., Th2 biomarkers and ADA)
- Photographs will be taken of the measured lesional and non-lesional sites for collection of skin swabs and tape strips. These sites should be the same areas that were selected and photographed during the

Treatment Initiation Visit. The areas swabbed should be the same size as the areas swabbed during the Treatment Initiation Visit, even if the lesion is smaller. Even if the lesion has cleared, the sample(s) should be labeled as “lesional”.

- Skin swab collection; 3 lesional and 3 non-lesional swabs to be used to determine *S. aureus* abundance, *S. aureus* superantigen, toxin, lipase, and protease quantitation; metagenomics, microbiome 16S rRNA analysis and skin microbiome functional assessment.
- Skin barrier assessments (TEWL basal and after 5, 10, and 15 tape strips)
- Skin tape strip collection; 1 set of 15 strips from non-lesional skin for lipidomics. These strips will be obtained as part of the TEWL barrier assessment.
- Skin tape strip collection; 1 set of 5 strips from lesional skin for lipidomics
- Assessment of AEs

Assessments and sample collection will be completed for all participants regardless of whether they used prohibited medications/procedures or met sampling criteria. Protocol deviations will be recorded, as applicable.

At the conclusion of this visit, participants will be allowed to resume use of prohibited medications/procedures as described in Section 7.3. Participants will be asked to continue to abstain from live (attenuated) vaccinations. They will be allowed to resume bathing/showering, use of chlorinated pools and hot tubs, and emollients without restrictions. Participants will be referred back to their primary care physician for their AD management. Sexually active participants of child-bearing potential will be instructed to use an acceptable method of contraception as described in Section 4.3.

Participants with asthma will be reminded that they should continue to take their asthma medications and will be counseled that they should only change their asthma medication in consultation with a physician. A letter will be provided to alert the physician managing their asthma that their patient is no longer participating in the clinical trial.

Participants will be provided with an instructional hand card including post-treatment reminders.

8.16. Day 182 Follow-Up Phone Call (\pm 5 days)

The purpose of the Day 182 Follow-Up Phone Call is to complete participant follow-up including assessment of concomitant medications, AEs, and pregnancy status. This call will be brief, and female participants of child-bearing potential will be asked if they have tested positive to a pregnancy test since their last visit. Male participants will be asked if their partner has tested positive to a pregnancy test since their last visit. Sexually active participants of child-bearing potential will be asked about birth control compliance and will be allowed to discontinue using an acceptable method of contraception as mandated by the protocol at the conclusion of this visit, should they elect to do so. Participants will be allowed to receive live (attenuated) vaccinations, as needed, after the conclusion of the Day 182 Follow-Up Phone Call. Participants with asthma will be reminded that they should continue to take their asthma medications and will be counseled that they should only change their asthma medication in consultation with a physician.

8.17. Unscheduled Visits

If disease activity increases or other concerns arise between regularly scheduled visits, participants should be instructed to contact study personnel and may be asked to return to the study site for an “Unscheduled Visit.” Participants may also be asked to return to the clinic for Unscheduled Visits, as needed, to provide additional blood, skin swabs, skin tape strips, and/or skin biopsies for further mechanistic and functional studies, or if samples are lost or destroyed, or if insufficient yields were obtained at a previous study visit.

Any of the following procedures, assessments, and laboratory measures may be conducted at the Unscheduled Visit:

- Interim medical history and physical examination, including assessments of AD severity (EASI, IGA, SCORAD, and Pruritus NRS)
- Confirm pregnancy status of female participants of child-bearing potential and partners of male participants, including performing a urine pregnancy test for female participants of child-bearing potential per investigator discretion
- Assessment of concomitant medications
- Vital signs, including temperature, blood pressure, heart rate, and respiratory rate
- Blood collection, up to 68 total mL, as applicable
- Photographs will be taken of the measured lesional and non-lesional sites for collection of skin samples, as applicable
- Skin swab collection, up to 6 total swabs, as applicable
- Skin tape strip collection, up to 15 non-lesional and 5 lesional tape strips, as applicable
- Skin biopsy collection, up to 2 total biopsies (1 x 3 mm non-lesional biopsy at [REDACTED], 1 x 2.5 mm lesional biopsy and/or 1 x 2.5 mm non-lesional biopsy at [REDACTED]s), as applicable
- IP Administration, as applicable
- IP Distribution, as applicable
- Assessment of AEs

At the conclusion of this visit, participants will be reminded to refrain from the use of prohibited medications/procedures as described in Section 7.3. Participants will be given instructions regarding restrictions on bathing/showering, use of chlorinated pools and hot tubs, and use of emollients, including instructions not to apply emollients to sampling areas 24 hours prior to study visits. Sexually active participants of child-bearing potential will be instructed to use an acceptable method of contraception as described in Section 4.3. Participants with asthma will be reminded that they should continue to take their asthma medications and will be counseled that they should only change their asthma medication in consultation with a physician. Participants will be reminded to contact the research clinic should they experience any adverse events, including new onset of eye symptoms.

8.18. Visit Windows

Study visits should take place within the time limits specified above. The designated visit windows (*i.e.*, $\pm n$ days) for each scheduled visit are also indicated on the Schedule of Events (Appendix A).

9. Mechanistic Assays

This section describes the proposed methodologies for this study. The techniques are state-of-the-art at the time of the writing of this protocol. Even so, the techniques will be updated and/or changed should there be additional technical breakthroughs in this area of research. The same methodologies/techniques will be utilized for all samples of a given type to ensure standardization and reduce variability. Details of the laboratory processes are described in Standard Operating Procedures (SOPs) maintained by each laboratory.

9.1. Measurement of *S. aureus* Abundance

Measurement of *S. aureus* abundance will be performed by the [REDACTED] laboratory at [REDACTED]. Skin microbiota will be collected by skin swabs from pre-measured areas on lesional and non-lesional skin. The swabs will be placed in cryotubes with sterile phosphate buffered saline (PBS) and stored at -80°C. Total DNA will be extracted from the suspended microbiota using a DNA isolation kit and a bead beater homogenizer for mechanical lysis. Genomic DNA will be quantitated by [REDACTED] and used for measurement of *S. aureus* abundance (as well as 16S

rRNA and metagenome analysis of the skin microbiome – refer to Section 9.3).

DNA extracted from skin swabs will be assayed by qPCR to quantitate the abundance of *S. aureus* at each collection site. Quantitation will be done using the *S. aureus* gene target (*femA*) in a multiplex [REDACTED] gene expression assay. A minimum of three technical replicates will be used for each participant sample. A standard curve of the *femA* PCR product will be used to quantify the *S. aureus* copy number or abundance.

9.2. Measurement of *S. aureus* Superantigens, Toxins, Lipase, and Proteases

Measurement of *S. aureus* superantigens, toxins, lipase and proteases will be performed by the [REDACTED] laboratory at [REDACTED]. Skin swabs will be frozen and batch shipped to the [REDACTED] laboratory. As part of the workflow for the measurement of *S. aureus* superantigens and toxins from skin swabs, staphylococci will be quantified by Colony-forming units (CFUs) of both *S. aureus* and coagulase-negative staphylococci by serial plate counts on mannitol salt agar. Yellow colonies will be tested by slide catalase and coagulase tests for verification as *S. aureus*. Red colonies will be verified as coagulase-negative by the same 2 tests. CoNS will be selected, inoculated into media, frozen, and shipped to the [REDACTED] laboratory. Premoistened skin swabs have approximately 0.1 mL liquid volume when wet. The swabs will be placed in 1.9 mL of saline for serial dilution. The lower limit of detection by this assay is 1 colony in the first 0.2 mL volume.

Proteins will be measured directly from swabs (0.6 total mL of the original 2 mL sample from above). Superantigens (staphylococcal enterotoxins A-G [excluding F which is now called TSST-1]), TSST-1, and enterotoxin-like superantigens H-X), cytotoxins (α , β , γ , δ , ϵ , and PSM-alpha3), lipase, and staphylococcal serine and cysteine proteases will be measured by Western Immunoblot. The Schlievert laboratory has on hand all required polyclonal hyperimmune antibodies to the native superantigens, cytotoxins, and lipase, and will have antibodies to the 2 major proteases by September 2019. For each sample, 20 μ L for assessment of each superantigen, cytotoxin, lipase, cysteine protease, and serine protease will be spotted directly and dried onto polyvinylidene fluoride (PVDF) membranes. At the same time, 3 serial, 10-fold dilutions of highly purified control superantigen, cytotoxin, lipase, and proteases will be treated similarly (the lower limit of detection of these toxins is approximately 75 pg). The PVDF membranes will be blocked with bovine serum albumin, reacted with 1/100 diluted primary antibodies, washed to remove unbound primary antibodies, reacted with 1/100 diluted conjugated antibodies against rabbit IgG (Li-Core IRDye 680 LT Goat Anti-Rabbit), washed again, and then developed. Reactions will be analyzed by NIH program Image J after scanning into the Odyssey CLx LI-COR instrument. A standard curve will be set up with the three dilutions of purified superantigens and cytotoxins. The reactivity of the saline samples will be compared for quantification.

9.3. Analysis of the Skin Microbiome

Analysis of the microbiome samples will be performed by the [REDACTED] laboratory at [REDACTED]. Skin swab samples obtained from non-lesional and lesional skin sites will be collected and stored at -80°C pending *S. aureus* abundance (see Section 9.1), 16S rRNA, and metagenome analysis.

9.3.1. Microbiome Assessment by 16S rRNA

Lesional and non-lesional skin swabs will be collected at all time points prior to and throughout dupilumab or placebo treatment. DNA extracted from skin swabs will be assayed by sequencing the V1-V3 16S rRNA hypervariable region to assess composition and diversity of the atopic dermatitis microbiome prior to and during treatment with dupilumab or placebo. The V1-V3 region will be amplified with PHusion High-Fidelity DNA Polymerase (New England Biolabs) using dual-indexed bar-coded primers (Fadrosh et al, 2014). Amplified products will be purified and normalized on [REDACTED] and pooled for 300 bp paired-end sequencing on an Illumina MiSeq. Quality and quantity of the libraries will be evaluated using an Agilent

BioAnalyzer. This approach routinely yields high quality sequence data, with ~40K reads per sample and assembly of 250-300 bp overlapping amplicons from the paired-end reads for each sample (Merkley et al, 2015).

9.3.2. Metagenomic Analysis of Microbiome

Metagenome analysis of skin samples collected prior to and during dupilumab or placebo treatment will identify genomic-level functional features of the microbiome. Metagenome libraries will be constructed using Illumina Nextera XT (Illumina) and sequenced on an Illumina HiSeq2500 using standard protocols for 125 bp paired-end sequencing. Samples will be multiplexed to provide a minimum of 80M mappable reads/sample. Based on ongoing metagenome analysis of atopic dermatitis samples, we anticipate that ~20% of the 80M reads will represent non-human or bacterial DNA. This will result in ~16M high-quality bacterial reads for metagenome analysis.

9.4. Functional Assessment of Coagulase Negative Staphylococcal Species (CoNS) Isolates for Antimicrobial Activity

Functional assessments for antimicrobial activity will be performed by the [REDACTED] laboratory at [REDACTED]. CoNS will be isolated from each swab sample by the Schlievert laboratory. CoNS strains will be stored at -80°C for future analysis.

Up to 84 individual colonies of CoNS will be randomly selected from each sample and transferred to TSB (400 µL) in a 96-well cluster tube for analysis. For analysis, each assay plate will contain internal controls of a non-antimicrobial strain of *S. epidermidis* (ATCC1457) as negative control, a known antimicrobial strain of *Staphylococcus hominis* producing Sh-lantibiotics as positive control, and blank wells without bacteria. CoNS clones will be expanded in a 96-well cluster tube at 37°C overnight with shaking at 250 rpm. Bacterial growth will be evaluated by measuring OD600 and only CoNS grown to (OD600>0.6) will be used for the following analysis. This will be performed by removing bacteria by centrifugation followed by sterile filtration by a 96-well filter plate with 0.22 µm PVDF membrane [REDACTED]. The antimicrobial activity in each sterile filtered media (100 µL) will then be evaluated by mixing with fresh TSB (10 µL) containing 1×10^4 CFUs of *S. aureus* (ATCC35556). Antimicrobial CoNS strains will be defined as those that suppress *S. aureus* growth after 22 hours to less than 50% (I50) of average growth seen in negative controls. The frequency of antimicrobial CoNS will be determined to total CoNS numbers subjected to the assay. CoNS isolates may then be processed for species identification by metagenomic sequencing.

9.5. Assessment of Lipid Components

Skin tape strip samples obtained from non-lesional and lesional skin sites will be collected and stored pending analysis.

The analyses of lipids including filaggrin breakdown products (urocanic acid [UCA] and pyroglutamic acid [PCA]) will be conducted at the [REDACTED] laboratory at [REDACTED]. Tape strips will be stored at -80°C until processed. Both lipids and UCA and PCA will be extracted from the skin tape strips and quantified using mass spectrometry. This protocol includes collecting biological skin specimens from skin tape strips, followed by a two-step modified Bligh and Dyer extraction with careful collection of both water-methanol and chloroform layers with a preservation of protein denaturants in the interphase for protein estimation. Biological material from skin tape strips #4 and #5 will be combined and processed. The material from skin tape strips will be removed by scraping in methanol/water (1:9, v/v) solution. The entire suspension will be subjected to chloroform/methanol extraction that allows extraction and separation of chloroform-soluble lipids and polar components. Protein denaturates will be digested overnight using 1N NaOH at 37°C; then protein content will be determined using BSA standards subjected to the same digestion procedure. The chloroform phase will be concentrated by a stream of nitrogen and redissolved in methanol for a subsequent liquid chromatography tandem mass spectrometry (LC-MS/MS) analysis of lipids. Separately, the water-methanol phase with polar components will be evaporated by a stream of nitrogen and redissolved in acetonitrile:water for a subsequent LC-MS/MS analysis. Each batch of skin tape strips will be accompanied by processing three blank tape strips to correct for background noise in each subsequent analysis.

Chloroform-soluble components (lipids) and water-soluble compounds (polar analytes, i.e. UCA and PCA) will be analyzed using reverse-phase chromatography or hydrophilic interaction chromatography (HILIC) and electrospray ionization tandem mass spectrometry (ESI-LC-MS/MS) on a Sciex 6500 QTRAP instrument interfaced with a Shimadzu Nexera X2 UHPLC system using developed targeted methods for the exact quantitation of analytes. Lipids will be separated on an Ascentis Express RP-amide column using gradient elution from System A (methanol:water:formic acid 65:35:0.5, with or without 5 mM ammonium formate) to System B (methanol:chloroform:water:formic acid 90:10:0.5:0.5, with or without 5 mM ammonium formate). UCA and PCA will be separated on a Waters Acquity UPLC BEH Amide column using gradient elution from System A (acetonitrile) to System B (methanol:water:formic acid, with 5 mM ammonium formate). Quantitation will be achieved by creating standard curves of responses of variable amounts of analytes versus fixed amounts of the internal standards. When the exact analyte is not available, quantitation parameters will be taken from the closest analog with the best possible approximation. To quantify UCA and PCA, 100 ng U-13C, 15N-proline will be added during the initial steps of sample processing. For quantitation of different groups of lipids, the following internal standards will be used: C13:0-lysophosphatidylcholine, N-12:0-sphingomyelin, D7-sphingosine, N-16:0-D7-sphingosine (D7-ceramide), and D7-cholesterol.

9.6. Confocal Imaging of Skin Biopsies

Confocal imaging will be performed by the [REDACTED] laboratory at [REDACTED]. Immediately after the non-lesional skin biopsy is obtained, the epidermis will be lifted from the dermis by a short incubation in dispase. For 2D imaging, fixed epidermal sheets will be stained for epidermal barrier proteins and innate immune receptors as well as markers of Langerhans cells (LC)/dendritic cells (DC) and markers of LC/DC activation as we have done previously for other ADRN studies (Kuo et al, 2013). We will use a laser scanning confocal microscope at the URMCC Confocal Microscopy Core. For 3D data visualization, processing, and analysis, we will utilize [REDACTED] software in order to more objectively capture the magnitude and localization of TJ molecules and innate receptors and their spatial relationship to LC/DC dendrites.

9.7. Skin Transcriptomics

Skin transcriptomic assays will be performed by the [REDACTED] laboratory at [REDACTED]. RNA-Seq transcriptomics of the skin before and during treatment will identify host transcriptional responses to dupilumab or placebo. Total RNA will be extracted from whole 2.5 mm skin biopsy samples using [REDACTED] according to the manufacturer's instructions. RNA will be quantitated by [REDACTED]. RNA integrity will be assessed on an Agilent BioAnalyzer. RNA-Seq libraries will be prepared using the [REDACTED]. [REDACTED] The double-stranded, tagged cDNA libraries will be purified using Agencourt AMPure Beads. Quality and quantity of the libraries will be evaluated using an Agilent BioAnalyzer. Sequencing will be performed on an Illumina HiSeq2500 using standard protocols for 100 bp paired-end sequencing. Samples will be multiplexed to provide a minimum of 20M mappable reads/sample for skin RNA. This approach will enable us to generate libraries from low-input RNA samples.

9.8. PBMC Immunoprofiling

Blood samples obtained for PBMC immunoprofiling will be collected and stored pending analysis.

PBMC immunoprofiling will be performed by the [REDACTED] laboratory at [REDACTED]. PBMC analysis of resting and activated populations will provide information on immune changes induced by IL-4 and IL-13 blockade by dupilumab. These changes may paradoxically include short-term increases in circulating Th2 and skin-homing T cells, as these cells are released from skin sites of inflammation, and/or inhibited from entering the skin. This data should be complementary to the transcriptomic analysis of skin biopsies. Longer-term immune changes may correlate with alterations in the microbiome, particularly the levels of *S. aureus*.

Peripheral blood samples will be collected at each clinical research site and shipped at room temperature to arrive at [REDACTED] within 24 hours. PBMCs will be prepared and cryopreserved. When all samples have been collected, the PBMC samples will be thawed and analyzed in batches, with all samples from a participant analyzed within the same batch to maximize the precision of intra-participant comparisons. Eighteen-color phenotyping panels will be used to identify resting leukocyte populations, as well as T cell responses to antigens and myeloid responses to TLR ligands. The panels will include analysis of T cell cytokines associated with Th2, Th17, and skin responses (IL-4, IL-13, IL-17, IL-22), as well as skin-homing subsets and myeloid response markers. Our new SWIFTreg registration program will be used to align samples between assay days to minimize any batch effects between different days.

9.9. Analysis of Serum Biomarkers

Serum biomarkers will be analyzed at a central laboratory, which will be determined for each biomarker. Th2 biomarkers to be assessed may include, but are not limited to, the following: thymus and activation-regulated chemokine (TARC/CCL17), pulmonary and activation-regulated chemokine (PARC/CCL18), total serum immunoglobulin E (IgE), and antigen-specific IgE. Serum may be used to measure ADA levels and other pharmacokinetic and pharmacodynamic studies.

9.10. Genotyping

Blood samples will be shipped to the Barnes lab at the [REDACTED] for DNA isolation. Isolated DNA samples will be pre-tested using an Illumina® Veracode TM 384-SNP barcode panel or a similar platform. The ancestry-informative markers-focused panel provides confirmation of the participant's gender, detects mendelian inconsistencies and duplicates in the sample set, assesses sample performance and produces a genetic barcode that allows samples to be tracked from production to data release. Samples that do not pass the barcode quality control will be excluded from further genetic studies. Extracted DNA samples may be genotyped on commercially available genome-wide association studies (GWAS) SNP chips, such as [REDACTED]

10. Biospecimen Storage

During the consent process, participants will be asked to give permission for long-term storage and future use of samples for research in the fields of AD and immunology. The following biospecimens will be stored:

- Serum
- PBMCs
- DNA, if collected
- Skin tape strip samples and any derivatives
- Skin biopsies and any derivatives
- Skin swabs and any derivatives

Instructions for sample preparation, handling, storage, and shipping are included in the MOP. Principal Investigators (PIs) will be responsible for being aware of and observing all the regulations for classification, packaging and labeling, permits or authorizations, and personnel training for shipment of biological and hazardous materials required for the conduct of this study.

11. Criteria for Participant and Study Completion and Premature Study Termination

11.1. Participant Completion

Participant participation will be defined as complete at the conclusion of the Day 182 Follow-Up Phone Call.

11.2. Participant Stopping Rules and Withdrawal Criteria

Participants may be prematurely terminated from the study for the following reasons:

1. The participant elects to withdraw consent from all future study activities, including follow-up
2. The participant is “lost to follow-up” (i.e., no further follow-up is possible because attempts to reestablish contact with the participant have failed). The ADRN standard definition of “lost to follow-up,” as defined in the MOP, will be used.
3. The participant dies
4. The participant becomes pregnant
5. The Investigator no longer believes participation is in the best interest of the participant
6. Medical condition for which continued participation, in the opinion of the Investigator, would pose a risk to the participant or would be likely to confound interpretation of the results
7. The participant has an anaphylactic reaction to study drug injection
8. The participant is diagnosed with a malignancy during study, excluding carcinoma in situ of the cervix, or squamous or basal cell carcinoma of the skin
9. The participant acquires any opportunistic infection, such as tuberculosis and other infections whose nature or course may suggest an immune-compromised status
10. The participant shows evidence of non-compliance to study protocol that in the opinion of the investigator requires discontinuation

11.3. Participant Replacement

Participants who were randomized and who withdraw or are withdrawn prior to the completion of the Day 182 Follow-Up Phone Call will not be replaced.

11.4. Follow-up After Early Study Withdrawal

If a participant is withdrawn from the study for any reason, the participant will be asked to complete a final visit by phone to assess any AEs and concomitant medications since their last visit. This visit will occur 30 days after withdrawal for any participant who withdraws before they receive study treatment and will occur 84 days after withdrawal if they have received study treatment (dupilumab or placebo). Any participant with an ongoing AE/SAE at the time of this contact will continue to be followed until the event is resolved with or without sequelae or until the AE stabilizes or until 30 days following this contact, whichever occurs first. Monitoring of a pregnant participant or consented partner of a male participant shall continue until the conclusion of the pregnancy.

11.5. Study Stopping Rules

Study enrollment will be suspended pending DAIT NIAID and NIAID Allergy and Asthma DSMB expedited review of all pertinent data if any of the following occur:

- 1 death, or life-threatening adverse event, that is possibly related to dupilumab
- A grade 3 or higher adverse event that is possibly related to dupilumab in two or more participants

The study may not be resumed until all pertinent information is discussed with DAIT NIAID, NIAID Allergy and Asthma DSMB, and the central IRB, and all parties concur with the resumption of the study. Local IRBs will be informed of the study stoppage and the DSMB/Central IRB's decision on resumption of the study.

The study may be terminated by DAIT/NIAID or the NIAID Allergy and Asthma DSMB upon review of any observations, events, or new information that merits such action.

12. Safety Monitoring and Reporting

12.1. Overview

This section defines the types of safety data that will be collected under this protocol and outlines the procedures for appropriately collecting, grading, recording, and reporting those data. AEs that are classified as serious according to the definition of health authorities must be reported promptly (per Section 12.5) to the sponsor DAIT/NIAID. Appropriate notifications will also be made to site PIs, and IRBs.

Information in this section complies with *International Conference on Harmonization (ICH) Guideline E2A: Clinical Safety Data Management: Definitions and Standards for Expedited Reporting*, *ICH Guideline E-6: Guideline for Good Clinical Practice (GCP)*, 21CFR Parts 312 and 320, and applies the standards set forth in the National Cancer Institute (NCI), Common Terminology Criteria for Adverse Events (CTCAE), Version 4.03: <http://ctep.cancer.gov/reporting/ctc.html>.

12.2. Definitions

12.2.1. Adverse Event (AE)

Any untoward or unfavorable medical occurrence associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research (modified from the definition of AEs in the most current ICH E-6 Guidelines for Good Clinical Practice) (from Office of Human Research Protections (OHRP) "Guidance on Reviewing and Reporting Unanticipated Problems Involving Risks to Subjects or Others and Adverse Events (1/15/07)" <http://www.hhs.gov/ohrp/policy/advevntguid.html#Q2>).

The investigator must report adverse events regardless of relationship to study therapy regimen or study mandated procedures.

For this study, an AE will include any untoward or unfavorable medical occurrence associated with:

- **Dupilumab or Placebo regimen:** An AE occurring after treatment initiation and within 84 days after the last dose of dupilumab or placebo
- **Study mandated procedures:**
 - **Blood Draw**

The following events related to the blood draw procedure will be considered AEs if they occur within 48 hours of the blood draw:

- Fainting / Vasovagal Events
- Bruising at the puncture site larger than 2 cm in diameter
- Bleeding from the puncture site lasting more than 30 minutes
- Swelling at puncture site larger than 2 cm
- Allergic reaction to topical anesthetic that requires use of rescue medications, detailed in Section 7.4

- **Tape Stripping**

The following events related to the skin tape strip collection procedure will be considered AEs if they occur within 48 hours of the tape stripping:

- Fainting / Vasovagal Events
- Bruising at the tape stripping site larger than 2 cm in diameter
- Bleeding from the tape stripping site lasting more than 30 minutes
- Redness or swelling at the tape stripping site larger than 3 cm
- Fever ($> 100.4^{\circ}\text{F}$) x two readings separated by more than 10 hours
- Allergic reaction to skin tape strips that requires use of rescue medications, detailed in Section 7.4

Purulent drainage from a tape stripping site within 2 weeks of the procedure will also be considered an AE.

○ **Skin Biopsy**

The following events related to the skin biopsies will be considered AEs if they occur within 48 hours of the skin biopsies:

- Fainting / Vasovagal Events
- Bleeding at skin biopsy site lasting more than 6 hours
- Redness or swelling at biopsy site larger than 2 cm in diameter
- Fever ($> 100.4^{\circ}\text{F}$) x two readings separated by more than 10 hours
- Allergic reaction to lidocaine that requires use of rescue medications, detailed in Section 7.4
Rescue Medications

Purulent drainage from a skin biopsy site within 2 weeks of the procedure will also be considered an AE.

12.2.1.1. Suspected Adverse Reaction (SAR)

Any AE for which there is a reasonable possibility that the study drug caused the AE. For the purposes of safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the product and the AE. A SAR implies a lesser degree of certainty about causality than adverse reaction, which means any AE caused by a drug (21 CFR 312.32(a)).

12.2.2. Unexpected Adverse Event

An AE or SAR is considered "unexpected" if it is not listed in the package insert or is not listed at the specificity, severity, or rate of occurrence that has been observed.

12.2.3. Serious Adverse Event (SAE)

An AE or SAR is considered "serious" if, in the view of either the investigator or DAIT/NIAID Medical Monitor, it results in any of the following outcomes (21 CFR 312.32(a)):

1. Death
2. A life-threatening event: An AE or SAR is considered "life-threatening" if, in the view of either the investigator or DAIT/NIAID Medical Monitor, its occurrence places the participant at immediate risk of death. It does not include an AE or SAR that, had it occurred in a more severe form, might have caused death.
3. Inpatient hospitalization or prolongation of an existing hospitalization
4. Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5. Congenital anomaly or birth defect

6. Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed above.

Elective hospitalizations are not to be reported as an SAE unless hospitalization is prolonged due to complications.

12.3. Grading and Attribution of Adverse Events

12.3.1. Grading Criteria

The study sites will grade the severity of AEs experienced by the study participants according to the criteria set forth in the NCI-CTCAE Version 4.03. This document (referred to herein as the NCI-CTCAE manual) provides a common language to describe levels of severity, to analyze and interpret data, and to articulate the clinical significance of all AEs. The NCI-CTCAE manual has been reviewed by the study investigators and sponsor and has been deemed appropriate for the participant population to be studied in this protocol.

AEs will be graded on a scale from 1 to 5 according to the following standards in the NCI-CTCAE manual:

Grade 1 = mild

Grade 2 = moderate

Grade 3 = severe

Grade 4 = life-threatening

Grade 5 = death

Events grade 1 or higher will be recorded on the appropriate AE electronic case report form (eCRF) for this study.

For grading an abnormal value or result of a clinical or laboratory evaluation (including, but not limited to, a radiograph, an ultrasound, an electrocardiogram etc.), a treatment-emergent AE is defined as an increase in grade from baseline or from the last post-baseline value that doesn't meet grading criteria. Changes in grade from screening to treatment initiation at Day 0 will also be recorded as AEs, but are not treatment-emergent. If a specific event or result from a given clinical or laboratory evaluation is not included in the NCI-CTCAE manual, then an abnormal result would be considered an AE if changes in therapy or monitoring are implemented as a result of the event/result.

For additional information and a printable version of the NCI-CTCAE manual, consult the NCI-CTCAE web site: <http://ctep.cancer.gov/reporting/ctc.html>.

12.3.2. Attribution Definitions

The relationship, or attribution, of an AE to the study drug or study procedure(s) will initially be determined by the site investigator and recorded on the appropriate AE eCRF. Final determination of attribution for safety reporting will be determined by the DAIT/NIAID Medical Monitor. The relationship of an AE to study therapy regimen or procedures will be determined using the descriptors and definitions provided in Table 12.3.2.

Table 12.3.2. Attribution of Adverse Events

Code	Descriptor	Relationship (to investigational products or study procedures: blood draw, skin tape stripping, skin biopsy)
NOT RELATED CATEGORY		
1	Not Related	The AE is clearly not related: there is insufficient evidence to suggest a causal relationship.
RELATED CATEGORIES		
2	Possible	The AE has a <u>reasonable possibility</u> to be related; there is evidence to suggest a causal relationship.
3	Related	The AE is clearly related.

12.4. Collection and Recording of Adverse Events

12.4.1. Collection Period

AEs will be collected from the time of consent until a participant completes study participation; or until 84 days after he/she prematurely withdraws (without withdrawing consent) or is withdrawn from the study if the participant received study treatment (dupilumab or placebo); or until 30 days after he/she prematurely withdraws (without withdrawing consent) or is withdrawn from the study if withdrawn prior to study treatment.

12.4.2. Collecting Adverse Events

AEs (including SAEs) may be discovered through any of these methods:

- Observing the participant
- Interviewing the participant in an objective manner [e.g., using structured questioning]
- Receiving an unsolicited complaint from the participant
- Receiving a call from the participant outside of their regular study visits; instructions for contacting the clinic will be provided on an instructional hand card
- In addition, an abnormal value or result from a clinical or laboratory evaluation can also indicate an AE, as defined in Section 12.3

12.4.3. Recording Adverse Events

Throughout the study, the investigator will record AEs and SAEs as described previously (Section 12.2) on the appropriate AE/SAE eCRF regardless of the relationship to study therapy regimen or study procedure.

Once recorded, an AE/SAE will be followed until it resolves with or without sequelae, or the AE/SAE stabilizes, or until the end of study participation, or until 30 days after the participant's final follow-up contact, whichever occurs first. Monitoring of a pregnant participant or consented partner of a male participant shall continue until the conclusion of the pregnancy.

12.5. Reporting of Serious Adverse Events and Adverse Events

12.5.1. Reporting of Serious Adverse Events to DAIT/NIAID

This section describes the responsibilities of the site investigator to report SAEs to DAIT/NIAID and the SACCC via the SAE eCRF. Timely reporting of AEs is required by 21 CFR and ICH E6 guidelines.

Site investigators will report all SAEs (see Section 12.2.3), regardless of relationship or expectedness within 24 hours of discovering the event.

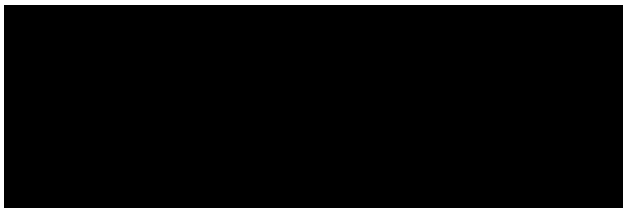
For SAEs, all requested information on the SAE eCRF will be provided. However, unavailable details of the event will not delay submission of the known information. Initial SAE eCRFs should include as much information as possible, but at a minimum must include the following:

- AE term
- Relationship to investigational product
- Relationship to study procedure
- Reason why the event is serious
- Supplementary CRF pages that are current at the time of SAE reporting: medical history, concomitant medications, demographics, investigational product administration

As additional details become available, the SAE eCRF should be updated and submitted. Every time the SAE eCRF is submitted, it should be electronically signed by the investigator or sub-investigator.

For additional information regarding SAE reporting, contact Rho Product Safety:

Rho Product Safety



12.5.2. Reporting to Health Authority

Not Applicable

12.5.3. Reporting of Adverse Events to the Central IRB

All investigators shall report AEs and SAEs, in a timely fashion to their local and central IRB in accordance with applicable regulations and guidelines.

12.6. Pregnancy Reporting

The investigator shall be informed of any pregnancy in a female study participant or the partner of a male study participant immediately upon becoming aware of the event. Study treatment will be discontinued for the pregnant participant. A male participant whose partner becomes pregnant will be allowed to continue study treatment. The investigator shall counsel the female participant or male participant with a pregnant partner and discuss the risks of continuing with the pregnancy and the possible effects on the fetus. Monitoring of the pregnant participant or consented partner of a male participant shall continue until the conclusion of the pregnancy.

The investigator shall report to the SACCC all pregnancies within 24 hours of becoming aware of the event using the Pregnancy eCRF. The SACCC will report all pregnancies to DAIT/NIAID. All pregnancies in female participants and

consented partners of male participants identified during the study shall be followed to conclusion, and the outcome of each must be reported. The Pregnancy eCRF shall be updated and submitted to the SACCC when details about the outcome are available.

Should pregnancy complications result in a congenital abnormality, birth defect, miscarriage, or medically indicated abortion - an SAE must be submitted to the SACCC using the SAE reporting procedures described in Section 12.5.

12.7. Reporting of Other Safety Information

An investigator shall promptly notify their local and central IRB, in accordance with applicable regulations and guidelines, as well as the SACCC and DAIT/NIAID via email when an “unanticipated problem involving risks to subjects or others” is identified, which is not otherwise reportable as an adverse event.

12.8. Review of Safety Information

12.8.1. Medical Monitor Review

The DAIT/NIAID Medical Monitor shall receive monthly reports from the SACCC compiling new and accumulating information on AEs, SAEs, and pregnancies recorded by the clinical sites on appropriate eCRFs.

In addition, the DAIT/NIAID Medical Monitor shall review and make decisions on the disposition of the SAE and pregnancy reports received by the SACCC (See Sections 12.5.1 and 12.6).

12.8.2. DSMB Review

The SACCC will provide the NIAID Allergy and Asthma DSMB with a listing of all AEs and SAEs on an ongoing basis (at least annually).

12.8.2.1. Planned DSMB Reviews

The NIAID Allergy and Asthma DSMB shall review safety data at least yearly during planned DSMB Review Meetings. Data for the planned safety reviews will include, at a minimum, a listing of all reported AEs and SAEs.

12.8.2.2. *Ad hoc* DSMB Reviews

In addition to the pre-scheduled data reviews and planned safety monitoring, the NIAID Allergy and Asthma DSMB may be called upon for *ad hoc* reviews when an event occurs that is of sufficient concern to the DAIT/NIAID Medical Monitor and/or the protocol chair to warrant DSMB review. The DSMB will be notified within 24-48 hours by the NIAID Medical Monitor and will promptly review any event that potentially impacts safety at the request of the protocol chair or DAIT/NIAID or any occurrence that meets the definition of the *Participant Study Stopping Rules* or *Study Stopping Rules* defined in Sections 11.2 and 11.5.

After review of the data, the DSMB will make recommendations regarding study conduct and/or continuation.

12.8.2.2.1. Temporary Suspension of Enrollment for *ad hoc* DSMB Safety Review

A temporary halt in enrollment will be implemented if an *ad hoc* DSMB safety review is required.

New participants will not be consented for study participation during the enrollment halt.

Participants already on therapy will continue dupilumab or placebo treatment. Participants screened but not yet randomized will not be allowed to continue with the Day 0 Treatment Initiation Visit. All participants not randomized within 12 days of the Screening Visit must rescreen.

13. Statistical Considerations and Analytical Plan

13.1. Overview

The primary research objective of this study is to assess the effect of dupilumab on *S. aureus* abundance on lesional skin as measured by microbial DNA. This objective will be addressed using a multicenter trial, involving a 6 week RDBPC portion followed by a 10 week OLE, investigating the effect of dupilumab treatment on quantitative and qualitative measures of cutaneous microbial community structure, skin barrier biology, and circulating T cell profiles for adult participants ages 18-75 years with chronic moderate-to-severe AD.

13.2. Endpoints/Outcomes

The primary endpoint is *S. aureus* abundance as measured by microbial DNA (*femA* qPCR) on lesional skin at Day 28.

Secondary endpoints include:

1. *S. aureus* abundance as measured by microbial DNA (*femA* qPCR) on lesional skin at Days 0, 3, 7, 14, 21, 42, 77 and 112
2. *S. aureus* abundance as measured by microbial DNA (*femA* qPCR) on non-lesional skin at Days 0, 3, 7, 14, 21, 28, 42, 77 and 112
3. Basal (prior to tape stripping) TEWL of non-lesional and lesional skin at Days 0, 3, 7, 14, 21, 28, 42, 77 and 112
4. TEWL area under the curve of non-lesional skin at Days 0, 7, 14, 21, 28, 42, 77 and 112. TEWL will be assessed prior to tape stripping and repeated after 5, 10, and 15 tape strips.
5. Change in TEWL per every 5 tape strips (i.e. slope) on non-lesional skin at Days 0, 7, 14, 21, 28, 42, 77 and 112
6. EASI, IGA, and SCORAD at Days 0, 3, 7, 14, 21, 28, 42, 77 and 112
7. Pruritus NRS at Days 0, 3, 7, 14, 21, 28, 42, 77 and 112

13.3. Measures to Minimize Bias

To minimize bias, randomization will be performed by the clinical sites using a SACCC validated system that automates the random assignment of treatment groups to study ID numbers. The randomization scheme will be reviewed by a statistician at the SACCC prior to implementation. Participants will be randomized using a 2:1 ratio of active (dupilumab) and control (placebo) participants. A stratified permuted block randomization algorithm will be used to maintain a balance between treatment arms with respect to clinical site and disease severity at Day 0 (EASI ≥ 21.1 or < 21.1).

13.4. Analysis Plan

13.4.1. Analysis Populations

The following groups of participants will define samples for endpoint analysis:

- Modified intent-to-treat (mITT) sample: All participants who are randomized and have *S. aureus* abundance measured at Day 0 and Day 28. Participants will be analyzed according to the treatment arm to which they were randomized, regardless of the medication they actually received.
- Safety sample: All participants who are randomized and receive at least one dose of study treatment. Participants will be analyzed according to the medication they actually received, regardless of the treatment arm to which they were randomized. Non-treatment emergent adverse events (e.g., any adverse event that occurs before the first injection) will be summarized in all participants who are enrolled, while treatment emergent adverse events (e.g., any adverse event that occurs on or after the first injection) will be summarized in the safety sample.

- Per-protocol (PP) sample: All participants who are randomized, receive the correct medication and dosage to which they were randomized, do not have a major protocol deviation, do not miss any scheduled injections, have not used any of the medications/procedures listed in Section 7.3 with the exception of topical calcineurin inhibitors (tacrolimus or pimecrolimus), topical corticosteroids, topical phosphodiesterase inhibitors (crisaborole) and topical antibiotics if applied to areas of the skin not being sampled, and have met sampling criteria.

13.4.2. Primary Analysis of Primary Endpoint(s)/Outcome(s)

The primary analysis will compare *S. aureus* abundance between treatment arms at Day 28 using an ANCOVA model applied to the modified intent-to-treat sample. Prior to analysis, a log-10 transformation will be applied to *S. aureus* abundance. If any participant to be included in the analysis has a value of zero, the participant's value will be adjusted by adding a value of 1 prior to transformation. To account for the study design induced by the randomization scheme, the model will also include fixed effects for clinical site and disease severity at Day 0, as measured by EASI ≥ 21.1 or < 21.1 . To increase statistical efficiency, the model will also adjust for skin swab collection site and *S. aureus* abundance at Day 0. Such a model will allow us to estimate the adjusted geometric mean ratio of the abundance of *S. aureus* at Day 28 (and corresponding 95% confidence interval) between the dupilumab and placebo arms. A two-sided significance level of 0.05 will be used for this analysis.

13.4.3. Supportive Analyses of the Primary Endpoint(s)/Outcome(s)

Analyses for the primary endpoint may be supported by several analyses.

To evaluate the distributional assumptions made in the primary ANCOVA model, a permutation test will be used as a supplementary analysis of the primary analysis (Manly, 1997).

The primary analysis will be replicated using *S. aureus* abundance as measured by microbial DNA (*femA* qPCR) on lesional skin at Day 28.

Rather than exclude participants who are randomized but do not have *S. aureus* abundance measured at Day 0 and Day 28 (as defined by the mITT sample), a multiple imputation technique will be employed to impute the missing primary endpoint of *S. aureus* abundance at Day 0 or Day 28. The ANCOVA model specified in Section 13.4.2 will be run on each imputed dataset, and results will then be pooled to estimate one geometric mean ratio and corresponding 95% CI.

The ANCOVA model specified in Section 13.4.2 will be adjusted to include clinical site as a random effect, rather than a fixed effect.

To test if the effect of dupilumab on *S. aureus* abundance at Day 28 is modified by particular sub-groups, separate ANCOVA models will be developed to test the two-way interaction between treatment arm and variables defining the sub-groups of interest. Sub-groups that will be investigated include:

- Clinical site
- Disease severity at Day 0, as measured by EASI ≥ 21.1 or < 21.1
- Disease severity at Day 0, as measured above and below the median value of TARC

13.4.4. Analyses of Secondary and Other Endpoint(s)/Outcome(s)

Generalized linear mixed models will be used to model the following endpoints at multiple time points: *S. aureus* on lesional and non-lesional skin (as measured by *femA* qPCR), basal TEWL on lesional and non-lesional skin,

TEWL AUC on non-lesional skin, change in TEWL on non-lesional skin, and disease severity, as measured by EASI (as well as percent change over time in EASI and EASI50), IGA (as well as percent change over time in IGA), SCORAD (as well as percent change over time in SCORAD) and Pruritus NRS (as well as percent change over time in Pruritus NRS). All models will be adjusted for clinical site, skin collection site, and disease severity at Day 0 as measured by EASI ≥ 21.1 or < 21.1 (for endpoints other than EASI). Data transformations (e.g., Box-Cox or arcsine square root) will be undertaken if the data deviate markedly from the normal distribution. For endpoints measured on both lesional and non-lesional skin, models will include a random effect for participant to account for correlation between lesional and non-lesional samples within the same participant. Likewise, skin collection site will also be considered as a random effect for endpoints collected on both lesional and non-lesional skin.

Analyses for the secondary endpoints may be supported by several analyses. For instance, to compare the cross-sectional and longitudinal effects of *S. aureus* abundance on disease severity between treatment arms, a generalized linear mixed model similar to that described in Chapter 15.4 of Fitzmaurice et al (2004) will be used. By including an interaction between treatment arm and *S. aureus* abundance at Day 0, as well as an interaction between treatment arm and the change in *S. aureus* abundance over time, this model will allow us to simultaneously estimate the effect of *S. aureus* abundance at Day 0 on disease severity at Day 0 in the dupilumab and placebo arms separately, as well as the change in *S. aureus* abundance over time on the change in disease severity over time in the dupilumab and placebo arms separately. The model will also be adjusted for clinical site, skin collection site, and disease severity at Day 0, as measured by EASI ≥ 21.1 or < 21.1 (for endpoints other than EASI).

13.4.5. Analyses of Exploratory Endpoint(s)/Outcome(s)

13.4.5.1. Skin Microbiome

13.4.5.1.1. 16S rRNA Analysis

Analysis of 16S rRNA microbiome data will identify changes in community composition and diversity at lesional and non-lesional skin sites prior to and throughout dupilumab or placebo treatment. The 16S rRNA bacterial sequence reads will first be assessed for quality and then analyzed using phylogenetic and Operational Taxonomic Unit (OTU) (Zhang et al, 2007) methods in QIIME (Caporaso et al, 2012). QIIME will be used to de-multiplex the barcoded reads and preprocess for quality. Chimera detection and removal will be performed *de novo* using UCHIME (Edgar et al, 2011). To identify OTUs, USearch 5.2 will be used to cluster the remaining reads based on percent identity, with a threshold of 97% in order to maximize taxonomic specificity (Edgar et al, 2010). Taxonomic assignments will be made to the resulting OTUs based on the seed of each cluster, using the RDP classifier with the GreenGenes database of reference sequences and their known taxonomic assignments (DeSantis et al, 2006). Within each sample type, individuals will be clustered based on constituent phyla using the hclust function of R, which performs hierarchical clustering iteratively, using the Lance-Williams dissimilarity update formula and computing complete distances. Bacterial diversity within each sample (alpha diversity) will be computed as the Shannon Index. Bacterial diversity between samples (beta diversity) will be computed as the Bray-Curtis dissimilarity index. Dissimilarity between lesional and non-lesional skin sites in each participant and treatment point will be assessed to correlate shifts in abundance of *S. aureus* and other members of the AD microbiome in response to dupilumab or placebo. In order to account for the correlation present in this experiment, we will analyze changes in *S. aureus* abundance using generalized linear mixed models. Between-group differences in overall microbiota composition will be visualized using unsupervised

machine learning methods, such as non-metric multidimensional scaling and principal components analysis.

13.4.5.1.2. Metagenomics

Comprehensive functional analysis of the AD microbiome will identify genomic level variation and changes in bacterial functions associated with dupilumab treatment. Sequence data will be processed for quality reads and those that map to human sequence. Taxonomic assignments will be made using reference based methods, including Bowtie (Langmead and Salzberg, 2012) and Pathoscope (Francis et al, 2013) with the Human Microbiome Project database of reference genomes. Functional gene groups will be characterized using the KEGG Orthology gene pathway (KO) (Abubucker et al, 2012; Kanehisa and Goto, 2000). Corresponding gene abundance will be calculated using the HMP Unified Metabolic Analysis Network (HUMANN) (Abubucker et al, 2012).

13.4.5.2. Skin Transcriptome

RNAseq reads will go through standard pre-processing procedures. We will align the short reads to the human reference genome using TopHat. Using Samtools, we will remove reads with low quality score or potential PCR duplicates. We will utilize the Cufflinks (Langmead and Salzberg, 2012; Kim et al, 2013; Trapnell et al, 2010) suite of software programs for discovery of differentially expressed genes between groups and novel splice variants. We will perform standard differential expression analysis, conditional quantile normalization correcting for GC content bias and employ surrogate variable approaches to identify, quantify, and account for potential artifacts in transcript level estimates (Hansen et al 2012; Leek et al, 2010; Leek and Storey, 2007). Functional enrichment will be performed using over-representation statistic and gene-set enrichment methods to find transcription factors (from TRANSFAC and JASPER) and molecular processes and pathways (from KEGG and GO databases) associated with dupilumab treatment (Yaari et al, 2013).

13.4.5.3. Epidermal Lipids

For each time point, we will compare lipid profiles between dupilumab and placebo arms as previously described (Janssens et al, 2012). A generalized linear mixed model, similar to that described in Section 13.4.4, will be used to investigate the effect of dupilumab on lipid profiles over time.

13.4.5.4. *S. aureus* Superantigens, Toxins, Lipase, and Proteases

We will compare the expression of each *S. aureus* superantigen (SAg), toxin, lipase, and protease over time between dupilumab and placebo arms using a generalized linear mixed model, similar to that described in Section 13.4.4. For the analysis, each endpoint will be considered in two ways: with continuous expression as the outcome, as well as a binary indicator for presence/absence as the outcome.

13.4.5.5. Functional Assessment of CoNS Antimicrobial Activity Data

The frequency of commensal Staphylococcus species producing antimicrobial activity, as measured by the number of colonies with antimicrobial activity against *S. aureus* per sample, between dupilumab and placebo will be compared using a generalized linear mixed model, similar to that described in Section 13.4.4.

13.4.5.6. PBMC Immunoprofiles

Flow cytometry analysis will be performed on PBMCs, using phenotyping panels to identify resting leukocyte populations, as well as T cell responses to antigens and myeloid responses to TLR ligands.

The flow data will be analyzed manually to conform with previous data, and also, with greater resolution, by algorithmic analysis by SWIFT (Naim et al, 2014; Mosmann et al, 2014). Algorithmic methods can often exceed the capabilities of manual methods (Aghaeepour et al, 2013), particularly in high dimensions. An extension of the main SWIFT algorithm, competitive template assignment (Rebhahn et al, 2016), further increases the resolution of SWIFT. This procedure was used in a blind analysis of PBMC samples from a dupilumab clinical trial, and successfully predicted the dupilumab group versus the placebo group, based on the differences between day 1 and day 29. In the ADRN Barrier study, the SWIFT competitive method identified multiple differences between non-atopic versus AD *S. aureus*- subjects, and also between AD *S. aureus*- and AD *S. aureus*+ subjects.

This initial analysis will be used to identify cell sub-populations and markers that distinguish the treatment groups. This can be a strong analysis, e.g., in a large systems biology analysis of vaccination, the flow data was the most discriminatory (Tsang et al, 2014). Refer to Section 13.4.5.8 regarding additional analysis of flow data.

13.4.5.7. Serum Biomarkers

We will compare serum biomarkers (Th2 biomarkers and ADA) over time between dupilumab and placebo arms using generalized linear mixed models, similar to that described in Section 13.4.4.

13.4.5.8. Systems Biology

The RNA-Seq data of host and microbiome will be integrated using statistical learning methods and graphical models (Thakar et al, 2013; Raghavan et al, 2007; Thakar and Albert, 2010; Campbell et al, 2011) to evaluate effects of host-pathogen interactions on microbial relative abundance, absolute *S. aureus* abundance, expression of *S. aureus* superantigens, toxins, lipase, proteases, AD severity, barrier function, and immune pathway activation upon dupilumab treatment. We have developed and used approaches to find co-regulated gene modules from transcriptomic data that are correlated with serum levels of the Th2 biomarker, TARC (Qian et al, 2014; Raghavan et al, 2007; Katanic et al, 2016). Furthermore, gene module approaches have been developed to adjust for confounder effects using multivariate regression to infer associations between host-pathogen networks and clinical parameters. We will also use machine learning algorithms, such as support vector machines and random forest, to find gene signatures that can discriminate between the dupilumab and placebo treatment groups in the RDBPC portion of this study (i.e. first 6 weeks) (Piepenbrink et al, 2016; Rebhahn et al 2016). To investigate the relationships between the gene-signatures and lipidomics specific to dupilumab treatment, we will extract features from lipidomics data and will integrate those with annotations using pathways databases. Thus, the host-pathogen networks will reveal molecular processes associated with AD severity, skin barrier function, and ultimately, dupilumab responsiveness.

We may complement the rich RNA expression and lipidomics data with high-resolution flow cytometry analysis (Refer to Section 13.4.5.6). The flow data will be combined with data on clinical responses, gene expression, lipidomics, and bacterial population changes in an integrated analysis of patterns that correlate with response and microbiome changes. The RDBPC portion as well as the OLE of the study will allow subject-specific functional analysis of the mechanistic readouts allowing us to identify signatures of intra-individual variation observed with fluctuation in clinical response (EASI, SCORAD, and NRS) in response to dupilumab treatment. Using both portions of this clinical study, we will identify associations between circulating immune cell populations (clusters), tissue gene-signatures, biogeography, and skin barrier functions including lipidomics that correlate with dupilumab responsiveness.

If genetic data are available, we will perform an exploratory, unbiased scan for quantitative trait loci (QTL) within each of the omics data types using genotype data generated using the MEGA chip imputed up to a reference panel (i.e., The 1000 Genomes Project) using conventional pipelines. We will consider both cis-QTLs and trans-eQTLs (defined at +/- 1Mb of gene start/stop) for eQTLs and microbiome QTLs (mbQTL) where the quantitative omics level can be linearly assigned to a gene window, but perform tests agnostic to a gene window for the mbQTLs. We will address the multiple comparisons problem and label QTLs as significant at FDR thresholds=5%.

13.4.6. Descriptive Analyses

Descriptive analyses will be reported separately for the dupilumab and placebo arms in the mITT, safety, and PP samples. Continuous baseline measures will be reported using a mean (or geometric mean) with 95% confidence interval or median with first and third quartiles, as appropriate. Categorical baseline and demographic characteristics and study disposition will be reported as frequencies and proportions.

13.5. Interim Analyses

13.5.1. Interim Analysis of Efficacy Data

No formal interim analysis of efficacy data will be performed for this study. However, statistical analyses of scientific objectives, to include all data through Day 112, are planned to occur as soon as applicable data collection is complete. The study database through Day 112 will be monitored and cleaned per the Data Management Plan. Data-entry for all study visits through Day 112 will be locked in order to begin analyses. Data-entry will remain open for safety data collected after Day 112, and this data will be analyzed once all study visits have been completed. Further details will be specified in the Statistical Analysis Plan.

13.5.2. Interim Analysis of Safety Data

The NIAID Allergy and Asthma DSMB will receive periodic safety reports on enrolled participants. However, no formal interim analysis of safety data will be conducted.

13.5.3. Futility Analysis

No formal futility analysis will be performed for this study.

13.6. Statistical Hypotheses

All primary and secondary objectives will be based on two-sided superiority tests. For instance, the null and alternative hypotheses for the primary objective are:

Null hypothesis: *S. aureus* abundance at Day 28 in the dupilumab arm is equal to *S. aureus* abundance at Day 28 in the placebo arm.

Alternative hypothesis: *S. aureus* abundance at Day 28 in the dupilumab arm is not equal to *S. aureus* abundance at Day 28 in the placebo arm.

Other secondary objectives have similar null and alternative hypotheses to the above.

13.7. Sample Size Considerations

The sample size for this trial is based on the primary endpoint of *S. aureus* abundance at Day 28. Based on previous data provided by [REDACTED] (Guttman-Yassky et al, 2016), the median (coefficient of variation) of *S. aureus* abundance at Day 28 after randomization was 108.9 (1.2) CFU/area and 1296.1 (2.2) CFU/area in the dupilumab and placebo arms,

respectively, corresponding to a ratio between the dupilumab and placebo arms of 0.08. Conservatively assuming that the coefficient of variation in each arm is 2.2 and the ratio of the medians will be an acceptable estimate for the geometric mean ratio between the two arms, a total sample size of 84 (56 in the dupilumab arm and 28 in the placebo arm) will allow us to detect a 0.36 geometric mean ratio of *S. aureus* abundance between dupilumab and placebo arms with at least 90% power via a two-sample pooled t-test of a mean ratio with lognormal data. The [REDACTED] study also showed that the percent change in EASI at 4 weeks was -63% (SD=29%) and the percent change in pruritus NRS at 4 weeks was -45% (SD=28%) in the dupilumab arm while in the placebo arm, the percent change in EASI at 4 weeks was 3% (SD=53%) and the percent change in pruritus NRS at 4 weeks was 7% (SD=79%). Assuming similar effects are seen in our study, this sample size will also allow us to detect these effect sizes with >99% and 91% power, respectively.

Assuming 15% of participants will not have *S. aureus* abundance available at Day 0 or Day 28 due to dropout or missing data, 99 participants will be randomized (66 in the dupilumab arm and 33 in the placebo arm) to ensure the sample size of 84 participants is met.

14. Identification and Access to Source Data

14.1. Source Data

Source documents and source data are considered to be the original documentation where participant information, visit consultations, examinations, and other information are recorded. Documentation of source data is necessary for the reconstruction, evaluation, and validation of clinical findings, observations and other activities during a clinical trial.

14.2. Access to Source Data

The site investigators and site staff will make all source data available to DAIT/NIAID and authorized representatives of DAIT/NIAID. Authorized representatives as noted above are bound to maintain the strict confidentiality of medical and research information that may be linked to identified individuals.

15. Protocol Deviations

15.1. Protocol Deviation Definitions

Protocol Deviation – The investigators and site staff will conduct the study in accordance to the protocol; no deviations from the protocol are permitted. Any change, divergence, or departure from the study design or procedures constitutes a protocol deviation. As a result of any deviation, corrective actions will be developed by the site and implemented promptly.

Major Protocol Deviation (Protocol Violation) - A Protocol Violation is a major deviation from the IRB approved protocol that may affect the participant's rights, safety, or well-being and/or the completeness, accuracy, and reliability of the study data. In addition, protocol violations include willful or knowing breaches of human subject protection regulations, or policies, any action that is inconsistent with the NIH Human Research Protection Program's research, medical, and ethical principles, and a serious or continuing noncompliance with federal, state, local, or institutional human subject protection regulations, policies, or procedures. Examples of Major Protocol Deviations are described in the MOP.

Non-Major Protocol Deviation - A non-major protocol deviation is any change, divergence, or departure from the study design or procedures of a research protocol that does not have a major impact on the participant's rights, safety, or well-being, or the completeness, accuracy, and reliability of the study data.

15.2. Reporting and Managing Protocol Deviations

The study site PI has the responsibility to identify, document, and report protocol deviations as directed by DAIT/NIAID. However, protocol deviations may also be identified during site monitoring visits or during other forms of study conduct review.

Upon determination that a protocol deviation has occurred, the study staff will a) notify the site PI, b) notify the SACCC and c) will complete a Protocol Deviation form. The protocol deviation form will document at minimum the date the deviation occurred, the date it was identified, a description of the event, whether the deviation resulted in an SAE/AE, PI signature, IRB report requirement, and documentation of a corrective action plan. DAIT/NIAID may request discussion with the site PI to determine the effect of the protocol deviation on the study participant and his/her further study participation, the effect of the protocol deviation on the overall study, and corrective actions. The PI will sign the paper source Protocol Deviation CRF, electronically sign Major Deviations in the electronic data capture (EDC), and submit the deviation to the central IRB, and local IRB/EC per IRB regulations. Major protocol deviations will be reported to the NIAID Allergy and Asthma DSMB by the DAIT/NIAID Medical Monitor at the medical monitor's discretion.

16. Ethical Considerations and Compliance with Good Clinical Practice

16.1. Quality Control and Quality Assurance

The investigator is required to keep accurate records to ensure that the conduct of the study is fully documented. The investigator is required to ensure that all CRFs are completed for every participant entered in the trial.

The sponsor is responsible for regular inspection of the conduct of the trial, for verifying adherence to the protocol, and for confirming the completeness, consistency, and accuracy of all documented data.

The CRFs will be completed online via a web-based EDC system that has been validated and is compliant with Part 11 Title 21 of the Code of Federal Regulations. Study staff at the site will enter information into the eCRFs, and the data will be stored remotely at a central database. Data quality will be ensured through the EDC system's continuous monitoring of data and real-time detection and correction of errors. All elements of data entry (i.e., time, date, verbatim text, and the name of the person performing the data entry) will be recorded in an electronic audit trail to allow all changes in the database to be monitored and maintained in accordance with federal regulations.

16.2. Statement of Compliance

This clinical study will be conducted using GCP, as delineated in *Guidance for Industry: E6 Good Clinical Practice Consolidated Guidance*, and according to the criteria specified in this study protocol. Before study initiation, the protocol and the informed consent documents will be reviewed and approved by the central IRB. Any amendments to the protocol or to the consent materials will also be approved by the central IRB before they are implemented.

16.3. Informed Consent Process

The consent process will provide information about the study to a prospective participant and will allow adequate time for review and discussion prior to his/her decision. The PI listed on the Investigator of Record or a designee will review the consent and answer questions. Consent designees must be listed on the site delegation of responsibilities log, complete consenting certification, and have demonstrated knowledge of the protocol and study procedures. The prospective participant will be told that being in the trial is voluntary and that he or she may withdraw from the study at any time, for any reason. The participant will read, sign, and date a consent form before undergoing any study procedures. Consent materials will be presented in the participant's primary language. A copy of the signed consent form will be given to the participant.

The consent process will be ongoing. The consent form will be revised when important new safety information is available, the protocol is amended, and/or new information becomes available that may affect participation in the study.

In the event that the partner of a male participant becomes pregnant, she will be invited to the research clinic to be consented into the study for pregnancy follow-up only. The consent process will be completed as outlined above.

Documentation of the consent process must be entered on a source document.

16.4. Privacy and Confidentiality

Following the Health Insurance Portability and Accountability Act guidelines, a participant's privacy and confidentiality will be maintained throughout the study. Each participant will be assigned a unique identification number and these numbers rather than names will be used to collect, store, and report participant information. All biological samples will be labeled with the identification number. Data reported in medical journals or scientific meetings will be presented in aggregate for participants as a whole. No individual participant will be identified in any way. Site personnel will not transmit documents containing personal health identifiers (PHI) to the study sponsor or their representatives.

17. Publication Policy

The ADRN Publications Policy will apply to presentations and publications of the results of this trial.

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Appendix A: Schedule of Events

Study Portion	RDBPC								Open-Label						Follow -Up	
	Recruitment	Screening Visit	Treatment Initiation (Day 0)	Day 3	Day 7	Day 14	Day 21	Day 28	Day 42	Day 56 Home IP Injection	Day 70 ± 7 ³ IP Injection	Day 77 ± 2	Day 84 Home IP Injection	Day 98 Home IP Injection	Day 112	
Study Visit		Day -28 to Day -8	Day 0	Day 3 ± 1 ²	Day 7 ± 2 ²	Day 14 ± 2	Day 21 ± 2	Day 28 ± 2	Day 42 ± 2	Day 56 ± 7 ³	Day 70 ± 7 ³	Day 77 ± 2	Day 84 Home	Day 98 Home	Day 112 ± 2	Day 182 ± 5
Visit Window																
Study Procedures/Evaluations																
Recruitment	X															
Informed Consent		X														
Demographics		X														
Participant Randomization			X													
Concomitant Medications		X	X	X	X	X	X	X	X			X			X	X
Medical History		X	X ⁴	X ⁴	X ⁴	X ⁴	X ⁴	X ⁴	X ⁴			X ⁴			X ⁴	X ⁴
Adverse Events		X	X	X	X	X	X	X	X			X			X	X
Pregnancy Status		X	X	X	X	X	X	X	X			X			X	X
Pregnancy Test ⁵		X	X			X		X	X			X			X	X
Vital Signs/Growth Parameters ⁶		X	X	X	X	X	X	X	X			X			X	X
Physical Exam		X	X ⁴	X ⁴	X ⁴	X ⁴	X ⁴	X ⁴	X ⁴			X ⁴			X ⁴	X ⁴
AD Severity Assessments ⁷		X	X	X	X	X	X	X	X			X			X	X
Blood Sample Collection																
CBC w/differential		X														X
Comprehensive Metabolic Panel		X														X
DNA ⁸			X													X
Serum Biomarkers			X		X	X	X	X	X						X	X
PBMC Immunoprofiling			X			X		X								X
Skin Sampling Location Photographs			X	X	X	X	X	X	X						X	X
Skin Swab Collection																
<i>S. aureus</i> Abundance (Les, NL)			X	X	X	X	X	X	X						X	X
Metagenomics (Les, NL)			X	X	X	X	X	X	X						X	X
Microbiome 16S rRNA analysis (Les, NL)			X	X	X	X	X	X	X						X	X

Study Portion	RDBPC							Open-Label						Follow -Up		
	Recruitment	Screening Visit	Treatment Initiation (Day 0)	Day 3	Day 7	Day 14	Day 21	Day 28	Day 42	Day 56 Home IP Injection	Day 70 Home IP Injection	Day 77	Day 84 Home IP Injection		Day 98 Home IP Injection	Day 112
Study Visit																
Superantigen, Toxin, Lipase, and Protease Quantitation (Les, NL)			X	X	X	X	X	X	X			X			X	X
Microbiome Functional Assessment ⁹ (Les, NL)			X	X	X	X	X	X	X			X			X	X
Skin Tape Stripping																
Lipidomics ¹⁰ (Les ¹¹ , NL)			X ¹¹		X	X ¹¹	X	X ¹¹	X			X			X ¹¹	X
Barrier Assessments ¹²																
Basal TEWL (Les, NL)			X	X	X	X	X	X	X			X			X	X
TEWL Area Under the Curve (NL)			X		X	X	X	X	X			X			X	X
TEWL Slope of the Curve (NL)			X		X	X	X	X	X			X			X	X
Skin Biopsy																
Confocal Imaging (NL) ¹³			X	X	X		X									X
Skin Transcriptomics ¹⁴ (Les, NL ¹⁵)			X ¹⁵	X ¹⁵	X ¹⁵		X									X
IP Administration			X ¹⁶			X ¹⁷		X ¹⁷	X ¹⁸	X ¹⁹	X ¹⁹		X ¹⁹	X ¹⁹		X
IP Distribution ²⁰									X			X				X

1. If medical concerns arise between regularly scheduled visits, participants will be instructed to contact study personnel and may be asked to return to the clinical site for an Unscheduled Visit. Participants also may be asked to complete an Unscheduled Visit for additional blood, skin swab, skin tape strip, or skin biopsy collection, as applicable, if samples are lost or destroyed, or insufficient yields were obtained during a previous visit or for further mechanistic and functional studies.
2. The Day 3 and Day 7 Visits should be scheduled a minimum of 2 days apart.
3. IP injections should occur at minimum 7 days apart. If a participant misses a dose it should be administered within 7 days from the missed dose. If the missed dose is not administered within 7 days, the participant will be instructed to wait until their next scheduled dose based on their original dosing schedule.
4. An abbreviated medical history and physical exam will be conducted at Days 0, 3, 7, 14, 21, 28, 42, 77, 112, and Unscheduled Visits.
5. A urine pregnancy test will be completed for all female participants of child bearing potential.
6. Temperature, blood pressure, heart rate, and respiratory rate will be measured. Growth parameters to include height and weight will only be measured at the Screening Visit.
7. AD disease severity will be assessed using the Eczema Area and Severity Index (EASI), Investigator Global Assessment (IGA), Nottingham Eczema Severity Score (NESS) (Treatment Initiation Visit only), SCORing Atopic Dermatitis (SCORAD), and pruritus Numerical Rating Scale (NRS).
8. DNA will only be collected from participants who did not have blood collected in a previous ADRN or ADVN study.
9. Samples for functional assessment of antimicrobial activity will be stored for future analysis.
10. All participants will have tape strips for lipidomics collected from non-lesional sites (1 set of 15/visit) at Days 0, 7, 14, 21, 28, 42, 77, and 112.
11. Tape strips for lipidomics from lesional sites (1 set of 5 strips) will only be collected at Days 0, 14, 28, and 112.

12. Tape strips for barrier assessments will only be collected from non-lesional sites. These strips will be used for the non-lesional lipidomics analysis.
13. Non-lesional skin biopsies (1x3 mm biopsy/visit) for confocal imaging will only be collected from participants at the [REDACTED] clinical site.
14. Non-lesional and lesional skin biopsies (1x2.5 mm biopsy each/visit) for skin transcriptomics analysis will only be collected from participants at [REDACTED] sites.
15. Non-lesional biopsies for skin transcriptomics will only be collected at Days 0 and 7.
16. Two 300 mg dupilumab or placebo subcutaneous injections (loading dose) will be administered in the abdomen, thighs, or upper arms. The two injections should be administered in different locations (e.g. 2 different abdominal quadrants).
17. One 300 mg dupilumab or placebo subcutaneous injection will be administered in the abdomen, thigh, or upper arm. The injection site should be rotated with each dose so the same area is not injected twice in a row.
18. Participants or their caregiver must administer the Day 42 injection(s) in the clinic at the Day 42 Visit if they intend to administer injections at home during the OLE. Study staff will confirm the participant and/or their caregiver is capable of administering injections at home during the OLE portion of the study. Participants originally randomized to the dupilumab group will receive one 300 mg dupilumab and one placebo subcutaneous injection. Participants originally randomized to the placebo group will receive a 600 mg loading dose (two 300 mg dupilumab subcutaneous injections).
19. The Day 56, 70, 84, and 98 IP injections (300 mg dupilumab) will be administered by the participant or their caregiver at home or at the study clinic if the participant is uncomfortable or unable to administer injections at home. The injection site should be rotated with each dose so the same area is not injected twice in a row.
20. IP will be distributed to participants on Day 42 for the Day 56 and 70 doses and on Day 77 for the Day 84 and 98 doses if they choose to administer injections at home.